A NEW SEPARATION METHOD USING AQUEOUS MIXED SOLVENTS AND AN OPEN TUBULAR CAPILLARY WITHOUT ANY SEPARATION COLUMN

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by

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ABSTRACT

In this research, the new separation method based on micro solvent cluster extraction and preferential solvation has been described. A proposed separation method using aqueous mixed solvents of organic solvents (acetonitrile, 1-propanol, 2-propanol and 1,1,1,3,3,3-hexafluoro-2-propanol) or ionic liquids and an open tubular capillary has been separated the model compounds (2-naphthol, 4-nitroaniline, phenol, 4-chlorophenol, 4-nitrophenol and 1,3,5-naphthalenetrisulfonic acid) sufficiently during their flow in a fused silica capillary tube (50 μm in I.D. and ca. 45 cm in length) without a specific separation column. For organic solvents, the separation power of the proposed method is due to salt enhanced micro-phase separation of the mobile phase between the capillary wall and the center of capillary tube. The model compounds have been also separated by using ionic liquids in water due to the formation of micro-solvent cluster formation of ionic liquid in the mixed solvent that was suggested by LAXS data. Moreover, the polymer added in the $BMIM^{+}Cl^{-}/H_2O$ mixed solvents enhances the aggregation of ionic liquid around the polymer in the mixed aqueous solution. The increased micro-solvent clusters of the ionic liquid increase theoretical plate number and separation factor. Aromatic compounds are concentrated in the ionic liquid clusters around and polar or ionic compounds are concentrated in the water phase

around the surface of capillary tube. Ionic compounds therefore elute slowly. The polymer ionic liquid, $C_{10}BMIM^{+}Cl^{-}$ also used to study the separation of model compounds and other polymer ionic liquids are in progress.

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CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

FIGURE **PAGE**

FIGURE **PAGE**

FIGURE **PAGE**

LIST OF ABBREVIATIONS AND SYMBOLS

AN	Acetonitrile
HFIP	1,1,1,3,3,3-hexafluoro-2-propanol
OTCLC	Open tubular capillary liquid chromatography
HPLC	High performance liquid chromatography
GC	Gas chromatography
CE	Capillary electrophoresis
GLC	Gas liquid chromatography
D_m	Diffusion coefficient of solute in mobile phase
D_s	Diffusion coefficient of solute in stationary phase
H_d	Longitudinal diffusion
H_s	Resistance to mass transfer in stationary phase
H_m	Resistance to mass transfer in mobile phase
d	Thickness of stationary phase
r_c	Radius of capillary
k'	Capacity factor
N	Theoretical plate number
I.D.	Internal diameter
SANS	Small angle neutron scattering
IL	Ionic liquid
MP	Melting point
UV-MALDI	Ultraviolet matrix-assisted laser desorption/ionization
MS	Mass spectrometry
RPLC	Reversed phase liquid chromatography
BGE	Background electrolyte
MEKC	Micellar electrokinetic chromatography
CZE	Capillary zone electrophoresis
t_R	Retention time of analyte
t_0	Retention time of unretained compound
a	Separation factor
w_b	Peak width
HETP	Height equivalent theoretical plate

L	Capillary length
TMS	Tetramethyl silane
mg	milligram
mL	milliliter
kg	kilogram
µm	micrometer
µg	microgram
SAXS	Small-angle X-ray scattering
min	Minute
nm	nanometer
v/v	volume/volume
Rs	Resolution factor
1,3,5-NTS	1,3,5-naphthalenetrisulfonic acid

CHAPTER I

INTRODUCTION

1.1 Introduction

This research describes a new separation method which utilizes the microheterogenities and preferential solvation phenomena using aqueous mixed solvents with a capillary tube. The micro-scale chromatography systems are more attractive than the conventional systems due to less sample injection and mobile phase consumption. We propose here a new type of open tubular, mechanically driven capillary liquid chromatography system which could overcome those limitations while preserving the merits, i.e., small volume of sample injection, less solvent consumption and high separation efficiency without using specific columns.

Recently, we have reported a new type of open tubular capillary liquid chromatography (OTCLC) which utilizes the microheterogenities and preferential solvation phenomena in acetonitrile-water mixed solvents.[1] Acetonitrile serves as a good example for these organic solvents. Under ambient condition acetonitrile is

miscible with water at any ratio; heterogeneities, however, have been observed at a molecular level in its water mixtures. It has been demonstrated that large acetonitrile molecular clusters as well as water molecular clusters coexist in the mixtures over a wide composition range.[2-5] The solvent clusters preferentially solvate to analyte depending on their solubility in acetonitrile and water. We call it microsolvent clusters extraction mechanism.[6] Such microheterogeneities could be further enhanced by addition of electrolytes as NaCl into the mixture.[7,8] When the salt concentration is high, phase separation occurs in the homogeneous acetonitrile-water mixture, resulting in an acetonitrile-enriched organic phase and a water-enriched aqueous phase. This salted-out two phases system serves as an effective platform for separation/extraction of inorganic as well as organic compounds which could not be extracted by conventional extraction systems.

In this research, the development of the OTCLC system is achieved upon the integration of a homemade splitter. The specially designed homemade "T" splitter was installed between the sample injection and the capillary tube. The splitting device is intensively needed for micro-scale chromatography to allow injection of very small amount of sample with minimal dilution of the sample band.

We will describe a new sepapration method without any separation column and we can change separation condition by choosing of water-soluble organic solvents and their composition.

1.2 Literature Reviews

Separation method is very important and essential in the fields of not only analytical chemistry but also environment sciences, organic synthesis, biology, pharmacology, medicine, and industries etc. Of separation methods High Performance Liquid Chromatography (HPLC)[9,10] is widely used in these fields and a lot of separation columns have been produced for analysis, but the composition of the inner columns is under veil of patents.

The proposed open tubular capillary liquid chromatography (OTCLC) system utilizes the recent discoveries of microheterogeneities in some organic-water mixed solvents and salting-out phase separation phenomena observed in these mixed solvents. Acetonitrile serves as a good example for these organic solvents. Under ambient condition acetonitrile is miscible with water at any ratio; heterogeneities, however, have been observed at a molecular level in its water mixtures. It has been demonstrated that

large acetonitrile molecular clusters as well as water molecular clusters coexist in the mixtures over a wide composition range.[2-5] The solvent clusters preferentially solvate to analytes depending on their solubility in acetonitrile and water. We call it microsolvent clusters extraction mechanism.[6] Such microheterogeneities could be further enhanced by addition of electrolytes as NaCl into the mixture.[7,8] When the salt concentration is high, phase separation occurs in the homogeneous acetonitrile-water mixture, resulting in an acetonitrile-enriched organic phase and a water-enriched aqueous phase. This salted-out two phases system serves as an effective platform for separation/extraction of inorganic as well as organic compounds which could not be extracted by conventional extraction systems.[3,4]

The inner wall of fused capillary is negatively charged due to the dissociation of its silanol groups. When an acetonitrile-water mixture with suitable salt concentration is pumped into such a capillary, micro-phase separation occurs near the capillary wall, resulting in a water-enriched aqueous phase attached to the capillary inner wall. In other words, a liquid membrane that has considerably different properties from the mobile phase is formed on the inner wall of the capillary. When a mixture of analytes is injected into the capillary, participation of the analytes occurs between the mobile phase and the liquid membrane, providing the separation of the analytes in the proposed

chromatography system.

The Golay equations[11] are readily applied to the proposed OTCLC system, and the height equivalent to a theoretical plate (HETP) is:

$$H = H_d + H_s + H_m$$

$$= \frac{2D_m}{u} + \frac{2k'd^2u}{3(1+k')D_s} + \frac{(11k'^2+6k'+1)r_c^2u}{24(1+k')^2D_m}$$

where H_d, H_s, and H_m stand for longitudinal diffusion and resistance to mass transfer in stationary and mobile phase, respectively. D_m and D_s are the diffusion coefficients of a solute in mobile and stationary phase. d is the thickness of the stationary phase and r_c is the radius of the capillary. k' is the capacity factor of the solute and u is the flow rate of the mobile phase.

For example, a theoretical study by Knox and Gillbert revealed that if an open tubular 10 μm capillary is used as separation column and the standard deviation of the un-retained solute is kept to less than 1 nL, a peak of N = 10^6 theoretical plate number will elute at 2 hours compared with 55 hours with a conventional packed columns.[12] Practicing liquid chromatographic separation with narrow tubing is, however, relatively

more difficult compared with its conventional counterparts. The difficulties in construction and operation of very narrow-bored capillary liquid chromatography are: (1) preparation of suitable stationary phase in the very narrow separation column and (2) injection and detection of extremely small sample volume. In order to increase the diffusivity and to decrease the viscosity of the mobile phase the increased temperature was applied on 50 μm I.D. column by Liu *et al.*[13] At 200°C, 1 million theoretical plates were obtained on a 19.6 m column in long. However, the stationary phase of commercially available columns is unstable under high temperature.

The separation of cations using OTCLC on 5 – 10 μm I.D. column was studied by Simon *et al.*[14] The capillary column was coated with the strong cation exchanger. To improve selectivity, OTCLC system usually used modified columns.[15,16]

Moreover, the open tubular capillary columns are widely used in capillary electrophoresis (CE).[17-19] The electronically driven CE is now achieving a typical several hundred thousands to millions number of theoretical plates and separates the analytes in as short as several seconds. However, there are some limitations of CE, such as is generally performed in aqueous solutions containing high electrolyte concentration assure a steady electronic current; the usage of high voltage (~ ten kv) post not only a concern in safety but also cause problems on the interface design when

the separation column is attached to other instruments such as mass spectrometry detector.

The separation principle is a micro-solvent cluster formation in mixed solvents[3-6] and a preferential solvation of solvent molecules to analytes in the mixed solvents.[7,8] Aqueous mixed solvents like the mixture of acetonitrile and water are homogeneous at macro scale but the mixtures are heterogeneous at micro scale. The microheterogeneity was directly observed by a small angle neutron scattering method (SANS) for the mixture of water and acetonitrile, tetrahydrofuran and 1,4-dioxane.[9] The SANS data indicated the solvent cluster formation, where water molecules assosciate with other water molecules and acetonitrile molecules associate with other acetonitrile molecules to form water clusters and acetonitrile clusters, respectively. The microheterogeneity increased by addition of NaCl.[6] Furthermore preferential solvation occurs to chemical species dissolved in aqueous mixed solvents and the phenomenon was confirmed by the measurement of change in fluorescence spectroscopy.[8] Compounds that are soluble in water are solvated preferentially by water molecules and compounds that are soluble in organic solvents are solvated preferentially by organic solvent molecules. Due to the two characteristics of mixed solvents, compounds dissolved in mixed solvents are “dissolved” or “extracted” in their

preferred solvent clusters, water or organic solvent cluster which depends on the chemical properties of the dissolved compounds. Therefore compounds spontaneously are separated in mixed solvents when dissolved in the mixed solvents. The idea has been explored to separation of chemical species using mixed solvents and a capillary without any specific separation column.[10] Compounds injected to a capillary separated during their flow in a capillary and eluted along with the difference of solubility of the compounds in micro-solvent clusters.

1.3 Objective

A new separation method based on micro solvent cluster extraction has been proposed. The water-soluble organic solvents and ionic liquids with different compositions have been used as mobile phase solutions for the separation of model compounds (2-naphthol, phenol, 4-chlorophenol, 4-nitrophenol and 1,3,5,naphthalenetrifonic acid) using an open tubular capillary.

CHAPTER II

THEORY

2.1 Theoretical Aspect

The proposed method open tubular capillary liquid chromatography system utilizes the microheterogenities in some organic-water mixed solvents and salting out phase-separation phenomena observed in this mixed solvents. Acetonitrile serves as a good example for these organic solvents. Under ambient condition, acetonitrile is miscible with water at any ratio; heterogeneities, however, have been observed at a molecular level in its water mixtures. It has been demonstrated that large acetonitrile molecular clusters as well as molecular clusters coexist in the mixtures over a wide composition range. The solvent clusters preferentially solvate to analytes depending on their solubility in acetonitrile and water. We call microsolvent cluster extraction mechanism. Such microheterogeneities were further enhanced by addition of electrolytes, such as NaCl, into the mixture. When the salt concentration is high, phase separation occurs in the homogeneous acetonitrile-water mixture resulting in an acetonitrile-enriched organic phase and water-enriched aqueous phase. This salted-out two phase system

serves as an effective platform for separation/extraction of inorganic as well as organic compounds which could not be extracted by conventional extraction systems.

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Preferential solvation of chemical species dissolved in aqueous mixed solvents was confirmed by the measurement of change in fluorescence spectroscopy, where compounds that are soluble in water are solvated preferentially by water molecules and compounds that are soluble in organic solvents are solvated preferentially by organic solvent molecules. Therefore, when chemical compounds are dissolved in aqueous mixed solvents, the compounds dissolve in the preferred solvent clusters, water or organic solvent cluster. This is "Microsolvent Cluster Extraction Mechanism" in aqueous mixed solvent.

The inner wall of a fused capillary is negatively charged due to the dissociation of its silanol groups. When an organic solvent-water mixture with suitable salt concentration is pumped into such a capillary, micro-phase separation occurs near the capillary wall, resulting in a water-enriched aqueous phase attached to the capillary inner

wall. In other words, a liquid membrane that has considerably different properties from the mobile phase is formed on the inner wall of the capillary. When a mixture of analytes is injected into the capillary, partition of the analytes occurs between the mobile phase and the liquid membrane, causing separation of the analytes in the proposed system.

2.2 Ionic Liquids[20]

Ionic liquids (ILs), also known as liquid organic, molten, or fused salts, are a class of nonmolecular ionic solvents with low melting points. The accepted definition of an IL is to be any salts that have a melting point lower than ambient temperature. However, ionic liquids are often applied to any compounds that have a melting point <100^{o}C. Most common ILs are composed of unsymmetrically substituted nitrogen-containing cations (e.g., imidazole, pyrrolidine, and pyridine) with inorganic anions (e.g., Cl^-, PF_6^-, BF_4^-). The chemical structures of some ionic liquids are presented in Table 1. The combination of such cations and anions can lead to a large number of ionic liquids that provide considerable flexibility in the selection of the most suitable pair for a specific chemical application.

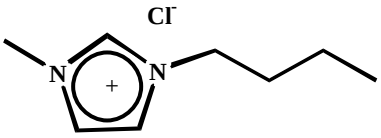
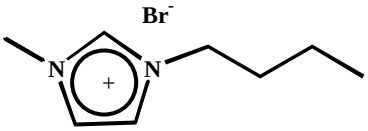
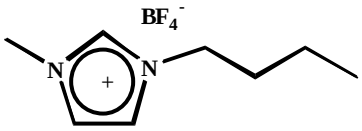
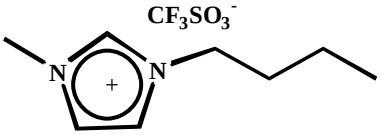
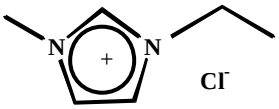
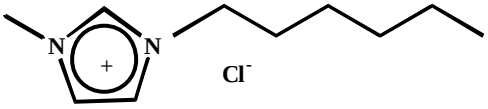
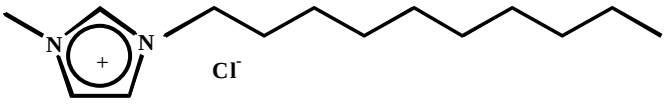
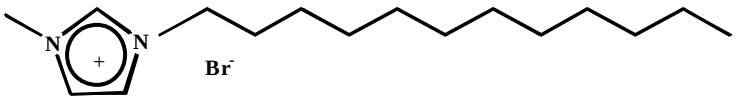
Chemical structure	Chemical name
	1-Butyl-3-methylimidazolium chloride [$BMIM^+Cl^-$]
	1-Butyl-3-methylimidazolium bromide [$BMIM^+Br^-$]
	1-Butyl-3-methylimidazolium tetrafluoroborate [$BMIM^+BF_4^-$]
	1-Butyl-3-methylimidazolium trifluoromethanesulfonate [$BMIM^+CF_3SO_3^-$]
	1-Ethyl-3-methylimidazolium chloride [$EMIM^+Cl^-$]
	1-Hexyl-3-methylimidazolium chloride [$HMIM^+Cl^-$]
	1-Decyl-3-methylimidazolium chloride [$C_{10}MIM^+Cl^-$]
	1-Dodecyl-3-methylimidazolium bromide [$C_{12}MIM^+Br^-$]

Figure 2.1 Chemical structures and chemical names of ionic liquids

Properties of ionic liquids[20]

The main properties of ILs are

(i) Under an inert atmosphere, they remain liquid over a temperature range of 200 to 300^{o}C;

(ii) They have practically no vapor pressure;

(iii) They are reported to have a wide window of electrochemical stability, good electrical conductivity high ionic mobility and excellent chemical stabilities.

With all these properties, it is hoped that they can act as “green solvents” and they will replace volatile organic in several chemical reactions. The properties of RTILs depend on the nature and size of both their cation and anion constituents. The physicochemical properties are presents in Table 2.

Table 2.2. Physicochemical properties of ionic liquids[21]

Ionic liquid	MP (°C)	Specific density (g/cm^3)	Surface tension (N/m)	Viscosity (cP)	Conductivity (S/m)
$BMIM^+Cl^-$	65	1080			
$BMIM^+Br^-$	78				
$BMIM^+BF_4^-$	-65	1120	0.0466	0.154	0.35
$BMIM^+CF_3SO_3^-$		1301		0.0844	0.29
$EMIM^+Cl^-$	89	1186			
$HMIM^+Cl^-$		1030	0.0425	0.716	
$C_{10}MIM^+Cl^-$	42				

Applications of ionic liquids

The ILs are used in chemistry such as: non-volatile solvents in organic synthesis, catalyzed reactions, electrochemistry and spectrometry. Many publications have been reported the uses of ionic liquids in analytical chemistry.

The uses of ILs in analytical chemistry include liquid phase and solid phase microextraction[22,23] as well as liquid-liquid extraction for separation metals and organic compounds[24-26], quartz crystal microbalance[27-30] for gas sensors and organic vapors, and

solvents for ultraviolet matrix-assisted laser desorption/ionization (UV-MALDI) mass spectrometry[31,32] (MS), ICP-AES. Many applications of ILs in electrochemistry[33-36] include photoelectrochemical solar cells, double-layer capacitors and electrodeposition of metal and alloys. The application range of ILs also extends to separation science: gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE).[9, 37]

The use of ILs as coated stationary phases for GC columns was an early analytical application in separation science. Research in this area has progressed with the advent of high temperature, crosslinked, and chiral IL GC capillary columns. Coated IL stationary phases used for the GC determination of physical chemistry parameters such as activity coefficient that quantify solute-IL interactions.[38-40] Solvation properties of ILs have been determined using the technique known as inverse GC. This technique uses generally packed columns with the interesting IL as the stationary phase into which selected test probes encompassing a wide class of volatile organic compounds are injected.

Ionic liquids, because of their good stability as liquids at both low and quite high temperatures, have been researched extensively as stationary phases for GLC.[41-43] Most recently, the synthesis and characterization of ILs as stationary phases for chiral GC separations has been reported. Both packed and capillary GC columns with IL stationary phases have been characterized for the separation of a wide variety of solutes with the intent to show selectivity differences as compared to more conventional GC stationary

phases. Strategies to improve the temperature stability of ILs include the synthesis of new ILs with bulky cationic or crosslinkable functionalities.[44,45] Higher temperature stable imidazolium ILs having different aromatic cations but the same anion such as 1-benzyl-3-methylimidazolium- and 1-(4-methoxyphenyl)- 3-methylimidazolium-tetrafluoromethane sulfonate were synthesized to expand the solvation capability to more classes of organic compounds.[44] The recent developments of immobilized IL GC stationary phases permitting high temperature work and chiral GC stationary phases will likely be extended.

The primary application of ILs in LC has been as mobile phase modifiers. However, recently research on the synthesis and characterization of covalently-bound LC stationary phases on silica has been initiated.[46,47] Ionic liquids have been used as stationary phase coatings for normal phase LC and covalently bound phases to silica for RPLC.[48]

Use of ILs as modifiers in the LC mobile phase has been reported that one of objective is to eliminate the need of organic modifier solvents in the mobile phase for RPLC generating a "green chemistry" LC method.[49] Because of the low volatility of ILs as compared to standard organic solvent modifiers, environmental containment is easier. Ionic liquids as mobile phase components can reduce peak tailing by masking residual silica silanols as well as affect solute retention through stationary phase and mobile phase

interaction.

Suppression of deleterious silanol effects due to incomplete silanization of silica-based packings can be done using 0.5-1.5 % levels of imidazolium tetrafluoroborates in the mobile phase. This class of ILs was found to markedly exceed the effectiveness of standard mobile phase suppressors such as alkylamines for the separation of basic drugs.[50] The nucleic acids, cytosine, cytodine, thymine, and two aromatic amino acids, tryptophan and phenylalanine derivatives were separated using various methanol – buffer mobile phase compositions with 1-butyl-3-methylimidazolium tetrafluoroborate varied from 0.5 to 15 mM using a commercially available C-18 column.[51] The separation of guanine and hypoxanthine was difficult even upon variation of the methanol – or acetonitrile – water composition in the absence of an IL.[52] An excellent separation was noted with the longer chain 1-octyl-3-methylimidazolium methylsulfate at the 2 mM level. The separation of octopamine, synephrine, and tyramine in citrus herbs using 1-ethyl-3-methylimidazolium tetrafluoroborate in a pH 4 aqueous mobile phase showed improved peak resolution and symmetry as compared to a mobile phase of only water adjusted to pH 4.[53]

Through addition of millimolar levels of ILs to the LC stationary phase, peak tailing of basic analytes by interaction with residual silanols can be alleviated and LC stationary phase modification by the hydrophobic imidazoliuum cation can affect solute retention. The use of ILs as major mobile phase components for the separation of

biological macromolecules such as proteins is a possible future application.

Use of undiluted ILs as mobile phase components at the high % level for RPLC is less common, as compared to standard organic solvents, due in part to the high viscosity and UV cut-off of ILs.

In general, ILs are typically used in CE as running electrolytes or modifiers. Similar to LC, their use in CE has shown to enhance resolution, peak efficiency, and peak symmetry mainly due to their binding to capillary wall, hence decreasing the unwanted effects of free silanols.[54-59] Furthermore, ILs can also be conveniently employed in CE at millimolar levels but at significantly higher millimolar concentrations than HPLC in both aqueous and nonaqueous running electrolytes. While the applications using GC have been on the physical properties of the volatile chiral and achiral analytes, in HPLC it is limited to polar organic compounds with no application to date on chiral separations. On the other hand, CE has shown great potential for broad range of compounds. This includes charged, multicharged, polar as well as nonpolar compounds. A variety of ILs that can be easily prepared according to published procedure or can be obtained from the chemical manufacturer were applied in CE as a: (i) single psedostationary phase or separation medium; (ii) binary pseudostationary in combination with micelles or CDs; (iii) support coating on the capillary wall; (iv) BGE in nonaqueous CE; (v) micelle forming surfactant for both achiral and chiral separations.[10]

Compared to MEKC and CZE, the use of ILs in CE has often been found to provide relatively shorter analysis time and higher separation selectivity. This is perhaps due to a relatively weaker intermolecular interaction, mediated by IL. Recently developed ILs with surfactant properties offer a large separation window and the size of the window can be controlled, hence offering greater separation potential for both hydrophobic neutral and charged compounds in MEKC. Continued emphasis on the use of ILs for chiral separations by CE is likely.[60,61]

Short-chain alkylimidazolium ionic liquids have found use in capillary electrophoresis as additives for both aqueous and non-aqueous background electrolytes. Recently, several studies deal with the behavior of long-chain alkylimidazolium ionic liquids as surfactants. However, only a few examples of the analytical utility of long-chain ionic liquids as self-assembled or as additives for MEKC separations have been presented.[60] The bulky organic ion of ionic liquids may induce a significant impact on the delicate balance between hydrophobic and electrostatic interactions within micelles leading to modifications of such characteristics as *e.g.* critical micelle concentration, aggregation number, micropolarity and surface charge. As part of the current intense research efforts to provide a better understanding of the several factors controlling the salt effect on micelles, several theoretical approaches have been developed, but their application is limited when the additive contains a large organic ion.

The aggregation behavior of ionic liquids in aqueous solution has been studied with variety methods such as ^{1}H NMR, steady-state fluorescence spectroscopy, refractometry, conductivity, surface tension measurement, *etc*.

2.3 Principle of Chromatography[61,62]

Chromatography is taken now to refer generally to the separation of components in a sample by distribution of the components between two phases – one that is stationary and one that move, usually but not necessarily in a column. Probably no other technique has been more valuable in the separation and analysis of highly complex mixtures.

Chromatographic processes can be classified according to the type of equilibration process involved, which is governed by the type of stationary phase. Various bases of equilibration are: (1) sorption, (2) solubility, (3) ion exchange, and (4) pore penetration.

The capacity factor ($k`$) is given by

$$k` = (t_R - t_0)/ t_0$$

where t_0 is the retention time of unretained compound and t_R is the retention of analyte.

Selectivity or separation factor (a) is a measure of the ability of the system to separate two solutes. Separation factor (a) is given by

$$a = k_2`/k_1`$$

Efficiency, N, is expressed as the number of theoretical plates, and can be calculated by measuring the migration time and the peak width, w_b, measured at the base of the peak:

$$N = 16\frac{t_R^2}{w_b^2}$$

Height equivalent theoretical plate (HETP) is given by

$$\text{HETP} = L/N$$

where L is capillary length (~ 450,000 μm).

CHAPTER III

EXPERIMENTAL

3.1 Chemical Reagents

3.1.1 1-Butyl-3-methylimidazolium chloride (TCI, Japan)

3.1.2 1-Ethyl-3-methylimidazolium chloride (TCI, Japan)

3.1.3 1-Hexyl-3-methylimidazolium chloride (TCI, Japan)

3.1.4 1-Butyl-3-methylimidazolium bromide (TCI, Japan)

3.1.5 1-Butyl-3-methylimidazolium tetrafluoroborate (TCI, Japan)

3.1.6 1-Butyl-3-methylimidazolium trifluoromethanesulfonate (TCI, Japan)

3.1.7 1-Decyl-3-methylimidazolium chloride

3.1.8 1-Dodecyl-3-methylimidazolium bromide

3.1.9 Methanol (Wako, Japan)

3.1.10 Acetonitrile (Wako, Japan)

3.1.11 1-Propanol (Wako, Japan)

3.1.12 2-Propanol (Wako, Japan)

3.1.13 Hexafluoropropanol

3.1.14 4-Nitroaniline (Wako, Japan)

3.1.15 2-Naphthol (Wako, Japan)

3.1.16 4-Chlorophenol (Wako, Japan)

3.1.17 Naphthalene (Wako, Japan)

3.1.18 Benzene (Wako, Japan)

3.1.19 Tolune (Wako, Japan)

3.1.20 Nitrobezene (Wako, Japan)

3.1.21 Polyvinylpyrrolidone (Wako, Japan)

3.1.22 Poly (N-isopropylacrylamide) (Aldrich)

3.1.23 Poly (benzyl methacrylate) (Aldrich)

3.1.24 Poly (styrene-co-methylmathacrylate) (Aldrich)

3.1.25 (Hydroxypropyl) methyl cellulose (Aldrich)

3.2 Instruments and Equipments

3.2.1 A syringe driver (Syringe Pump Controller MF-9090, 0.1 μdm^3, BAS, U.S.A.)

3.2.2 A syringe 1 mL (Hamilton Gastight)

3.2.3 A microsample injector 0.5 μL (Model 7520, Rheodyne, U.S.A.)

3.2.4 Fused silica capillary tube with an I.D. of 10 μm and and O.D. of 375 μm (Jasco, Japan)

3.2.5 An intelligent UV-vis detector of capillary (CE-970, JASCO, Japan)

3.2.6 A computer with LC-NET

3.2.7 Volumetric flasks 5,10, 25, 50 and 100 mL

3.2.8 Beakers 10 and 25mL

3.2.9 An analytical balance

3.2.10 Micropipette

3.3 Chromatographic conditions

The separation system consists of a syringe driver (Syringe Pump Controller MF-9090 with adjustable flow rate at 0.1 μdm^3 (μdm^3= µL)/min to 100 µL/min, BAS, USA) together with a Hamilton Gastight 1 cm^3 (cm^3= mL) syringe for pumping mobile phase solvent. A 0.5 µL micro-sample injector (Model 7520, Rheodyne, USA) linked with a homemade "T" splitter was used for sample injector and a fused silica capillary tube with I.D. 50 µm and O.D. 375 µm of ~45 cm in length (from the injection port to the detection window). An intelligent UV/Vis detector of capillary (CE-970, JASCO, Japan) was used for detection. The detector was connected to a personal computer through JASCO LC-NET. Data collection and chromatogram analysis were accomplished by JASCO-Borwin®. The concentrations of analytes were 2.5 mM.

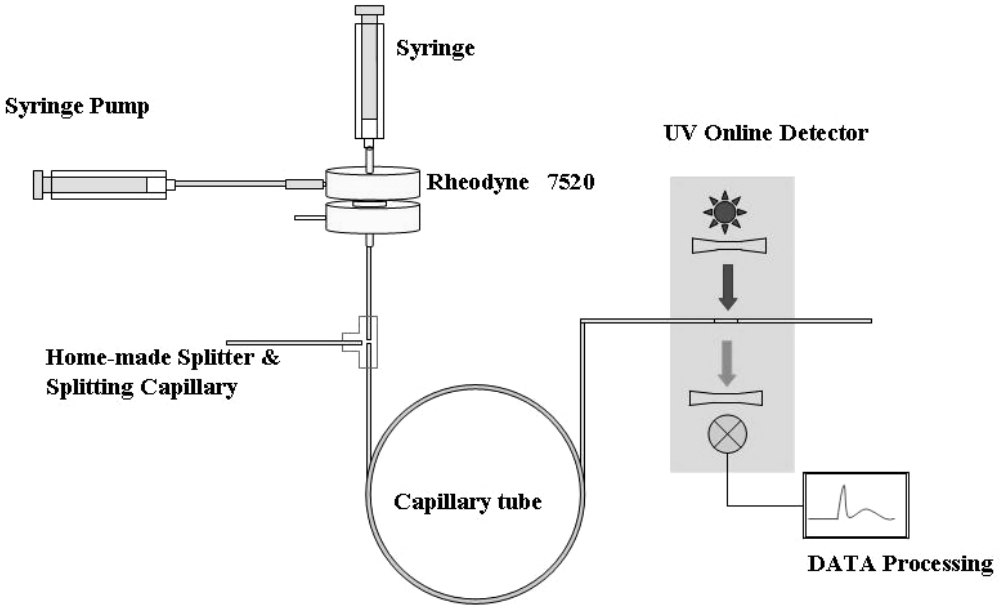


Figure 3.1 Layout of open tubular liquid chromatography

Ionic liquid solutions mixed with water at different concentrations were used as mobile phase. Stock solutions of 2-naphthol, phenol, 4-chlorophenol, 4-nitrophenol, and 1,3,5-naphthalenetrisulfonic acid were prepared by dissolving each compound in a small amount of methanol and the mixture of 2-naphthol, phenol, 4-chlorophenol, 4-nitrophenol, and 1,3,5-naphthalenetrisulfonic acid was prepared and diluted to 2.5 mM with the mixed solvent of water and $BMIM^+Cl^-$ that was used as a mobile phase. All chromatograms were obtained at room temperature and at a flow rate 1 μL min^{-1}. The void volume, t_0, of the capillary was determined by the used of methanol. UV detection at 223 nm was used throughout the experiment. Before injection of samples the capillary was washed successively with 0.1 M NaOH for 30 min and water for 30 min. For the use of different concentrations of ionic liquid, the capillary was washed again with water and 0.1 M NaOH for 30 min, respectively and

followed by water for 60 min to confirm a complete same experimental condition in capillary tube.

3.4 Measurement of E_T^N values[64]

$E_T(30)$ value is know as a solvent polarity parameter based on $\pi-\pi^*$ charge transfer at the wavelength of the maximum absorption band DTP (dye No. 30). The $E_T(30)$ values for samples are determined by the following equation

$E_T(30) = 1.196 \times 10^5 \times l^{-1}$/kJ mol^{-1}

where l denotes the wavelength (nm) of the observed absorption maximum. The normalized E_T^N value has also often been used instead of the $E_T(30)$ value. The E_T^N value is defined by extreme reference solvents of water and tetramethyl silane (TMS) as follows:

$E_T^N = (E_T(30)_S - E_T(30)_{TMS}) (E_T(30)_W - E_T(30)_{TMS})^{-1}$,

where $E_T(30)_S$, $E_T(30)_W$ and $E_T(30)_{TMS}$ are the values for the sample, water (264 kJ mol^{-1}) and TMS (128 kJ mol^{-1}), respectively. The UV visible absorption spectra of samples were measured at room temperature on a UV visible spectrometer (2200A, Shimadzu Co., Kyoto, Japan.).

The concentration of each ionic liquid at 0.5 M was prepared in water.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 The separation of organic compounds using organic solvents

The proposed system was employed to study model compounds (2-naphthol and 4-nitroaniline) by using many organic solvents such as acetonitrile, 1-propanol, 2-propanol and 1,1,1,3,3,3-hexafluoropropan-2-ol (HFIP). Table 4.1 shows physicochemical properties of organic compounds which used in this research. These organic solvents have been studied in microheterogeneity and preferential solvation in our research group. The physicochemical properties of organic solvents which used in this research are shown in Table 4.1.

Table 4.1 The physicochemical properties of organic solvents

solvent	Boiling point (°C)	ΔG_h° [64]	log P_{OW}
acetonitrile	81.6	-16.26	-0.34
1-propanol	97.2	-20.19	0.25
2-propanol	82.0	-19.90	0.05
HFIP	58.2	-15.77	

ΔG_h° : Free energy of hydration at 25°C; log P_{OW} : Partition coefficient

Figure 4.1 shows that decreasing of acetonitrile in mobile phase slightly increases the elution time of 2-naphthol and 4-nitroaniline. Moreover, the absence of NaCl in the mobile phase, no separation was observed while the presence of NaCl the model compounds could separate as presented in Figure 4.5.

The separation of model compounds was also studied by other organic solvents. Figure 4.1 - 4.4 shows the chromatograms of separation when using different composition of organic-aqueous mixed solvents. The results show that decreasing of organic solvents in mobile phase slightly increased the elution time of both model compounds. By the way, the presence of NaCl in organic-aqueous mixed solvent as shown in Figure 4.5 – 4.8 the model compounds could significantly separate, and the chromatographic parameters are presented in Table 4.4. The results when using

2-propanol/H_2O aqueous mixed solvent as mobile phase are almost the same as using 1-propanol. When using acetonitrile, 1-propanol and 2-propanol the addition of electrolytes for the micro-phase separation in capillary tube is necessary.

The results when using HFIP- H_2O aqueous mixed solvent as mobile phase gave in Figure 4.4, and the chromatographic parameters are shown in Table 4.3. The model compounds could separate in the absence of NaCl due to the low polarity of HFIP allows the formation of micro clusters in aqueous solution. The evidence for microheterogeneity in HFIP water mixtures was studied by small-angle X-ray scattering (SAXS).[65]

Table 4.2 The physicochemical properties of organic compounds[66]

Organic compounds	M.W.	pKa
2-naphthol	144.1699	9.46
4-nitroaniline	138.1240	
phenol	94.1112	9.95
4-chlorophenol	128.5563	9.38
4-nitrophenol	139.1088	7.08
1,3,5-NTS	434.31	

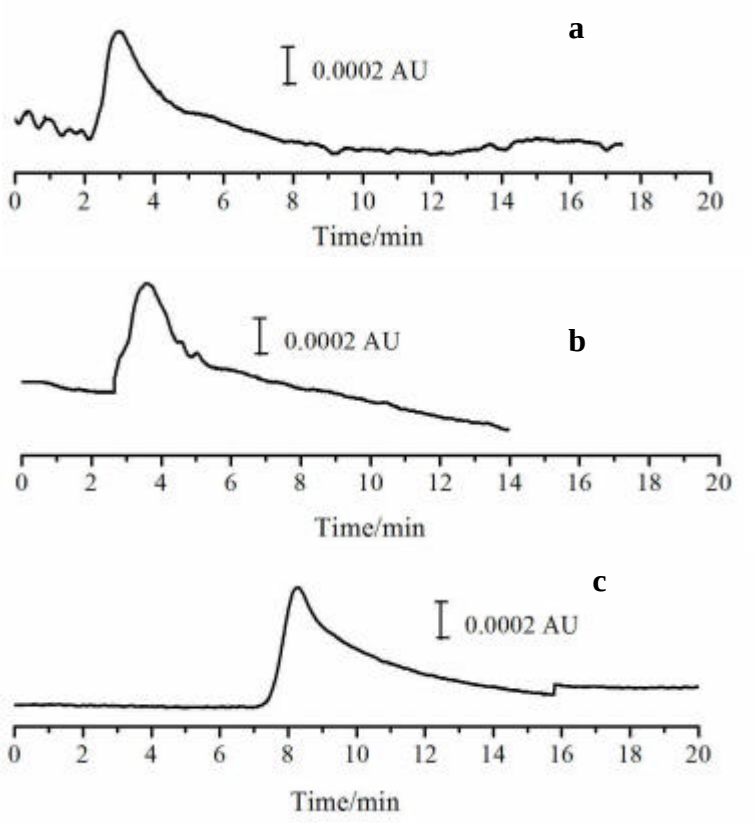


Figure 4.1 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different composition of AN/H_2O: (a) 8/2, (b) 7/3, and (c) 6/4 (v/v). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection limit 254 nm; room temperature.

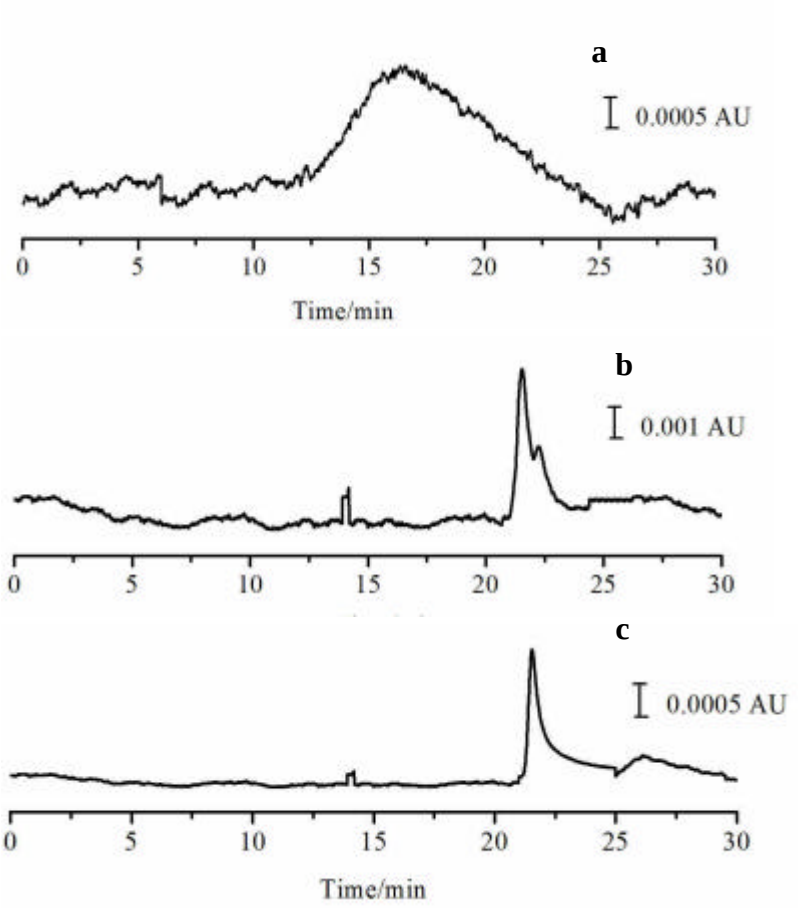


Figure 4.2 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different composition of 1-propanol/H_2O: (a) 8/2, (b) 7/3, and (c) 6/4 (v/v). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection limit 254 nm; room temperature.

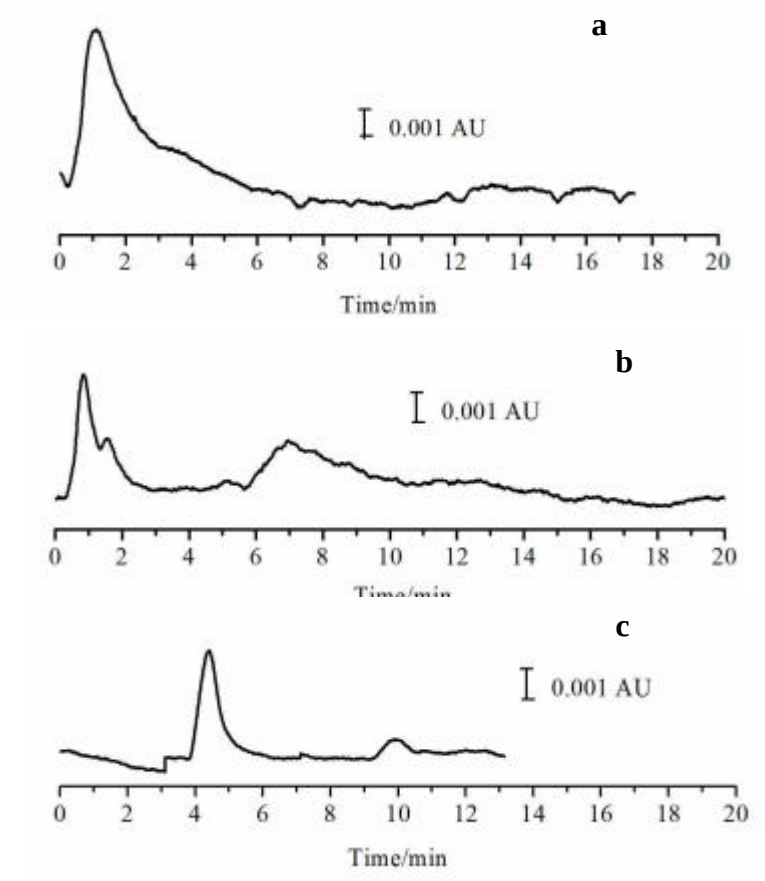


Figure 4.3 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different composition of 2-propanol/H_2O: (a) 6/4, (b) 5/5, and (c) 2/8 (v/v). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection limit 254 nm; room temperature.

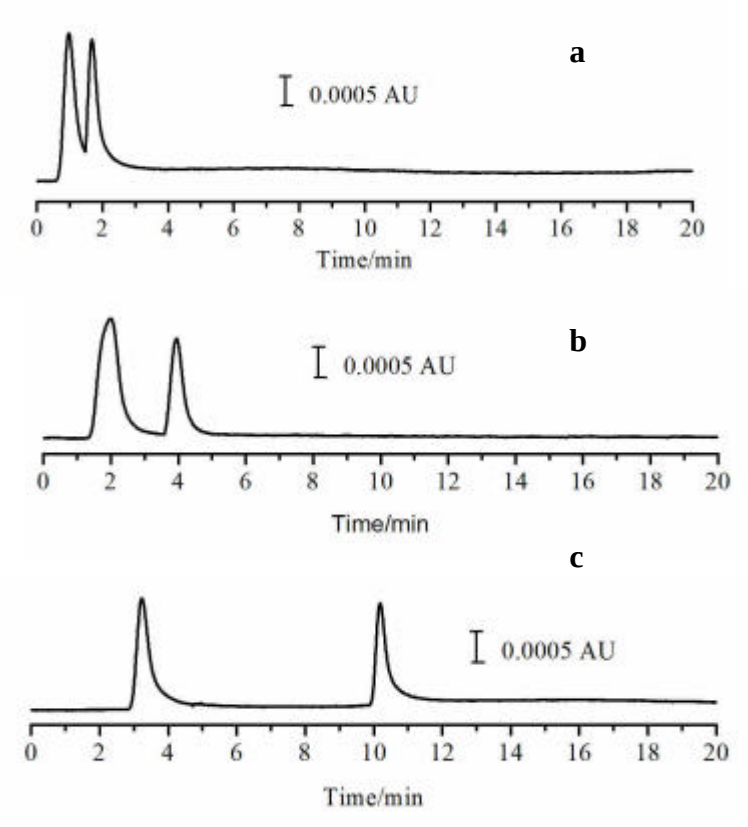


Figure 4.4 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different composition of HFIP /H_2O: (a) 8/2, (b) 7/3, and (c) 6/4 (v/v). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection limit 254 nm; room temperature.

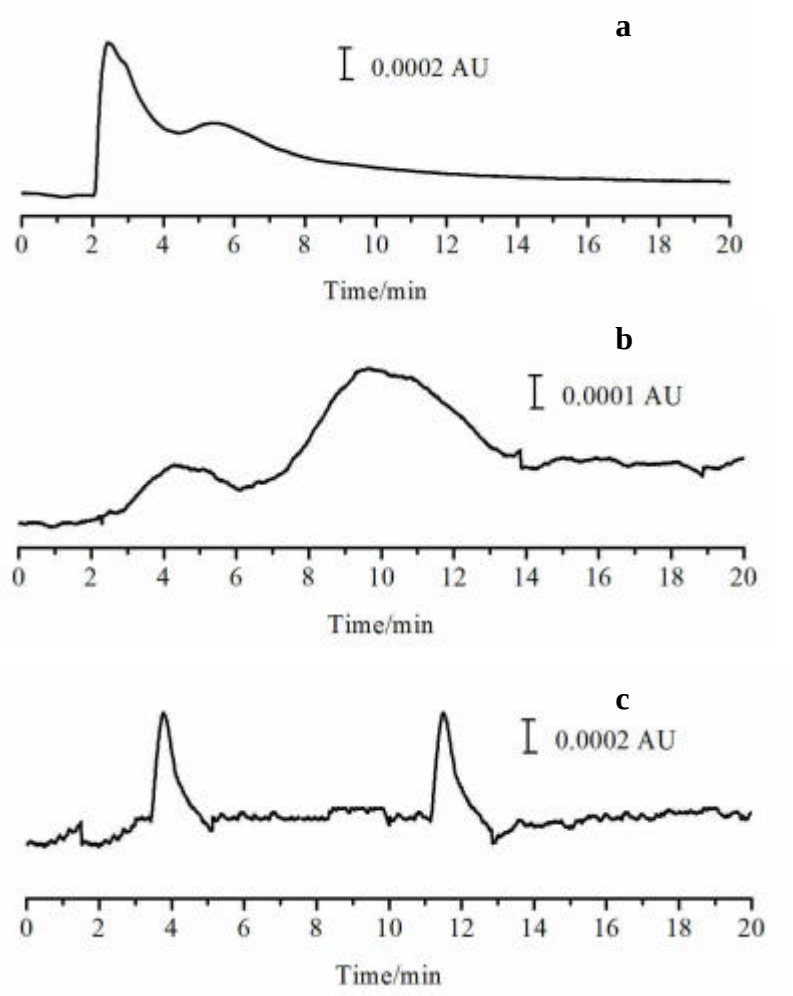


Figure 4.5 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different composition of AN/H_2O in the presence of 0.1 M NaCl: (a) 8/2, (b) 7/3, and (c) 6/4 (v/v). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection limit 254 nm; room temperature.

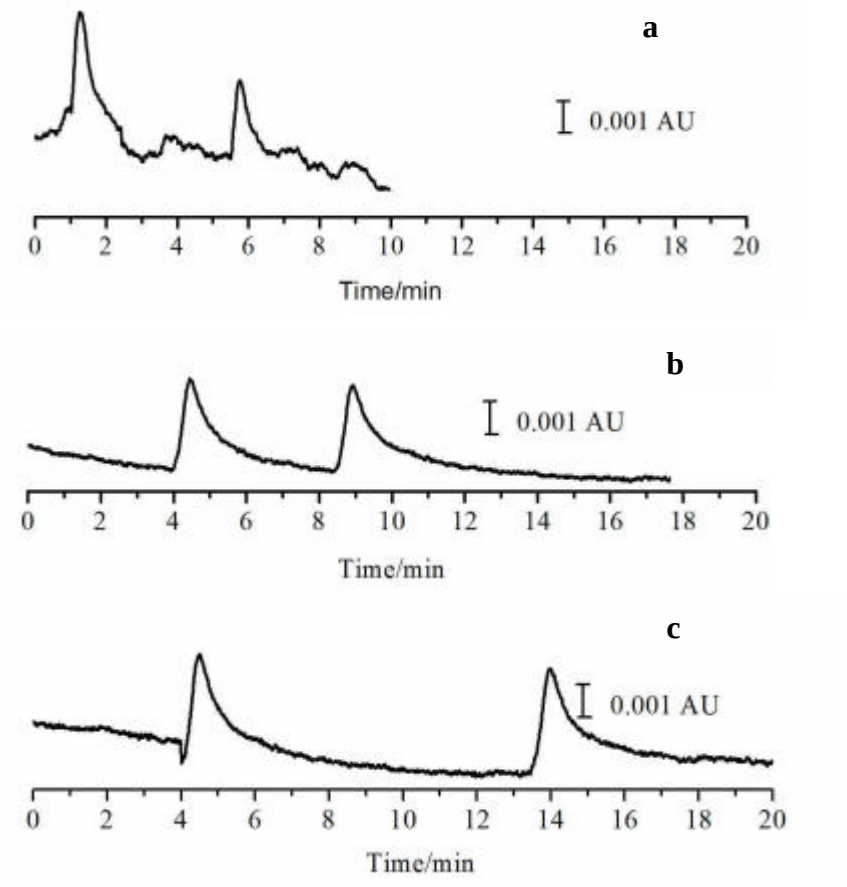


Figure 4.6 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different composition of 1-propanol/H_2O in the presence of 0.1 M NaCl: (a) 8/2, (b) 7/3, and (c) 6/4 (v/v). Chromatographic conditions: flow rate: 1 µL min^{-1}; detection limit 254 nm; room temperature.

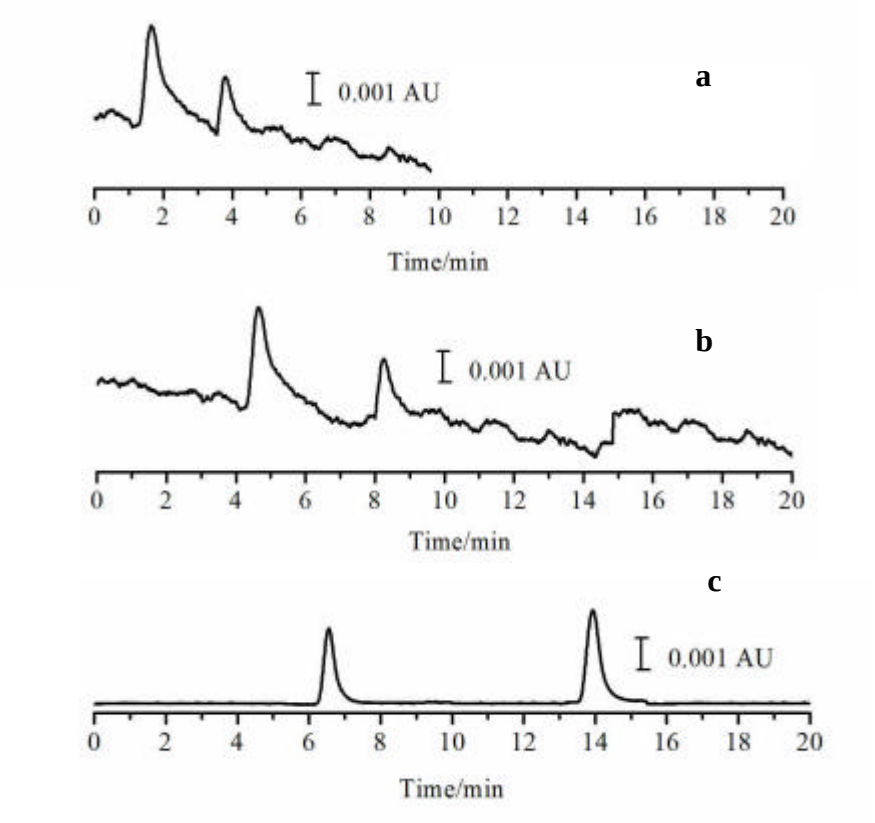


Figure 4.7 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different composition of 2-propanol/H_2O in the presence of 0.1 M NaCl: (a) 6/4, (b) 5/5, and (c) 2/8 (v/v). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection limit 254 nm; room temperature.

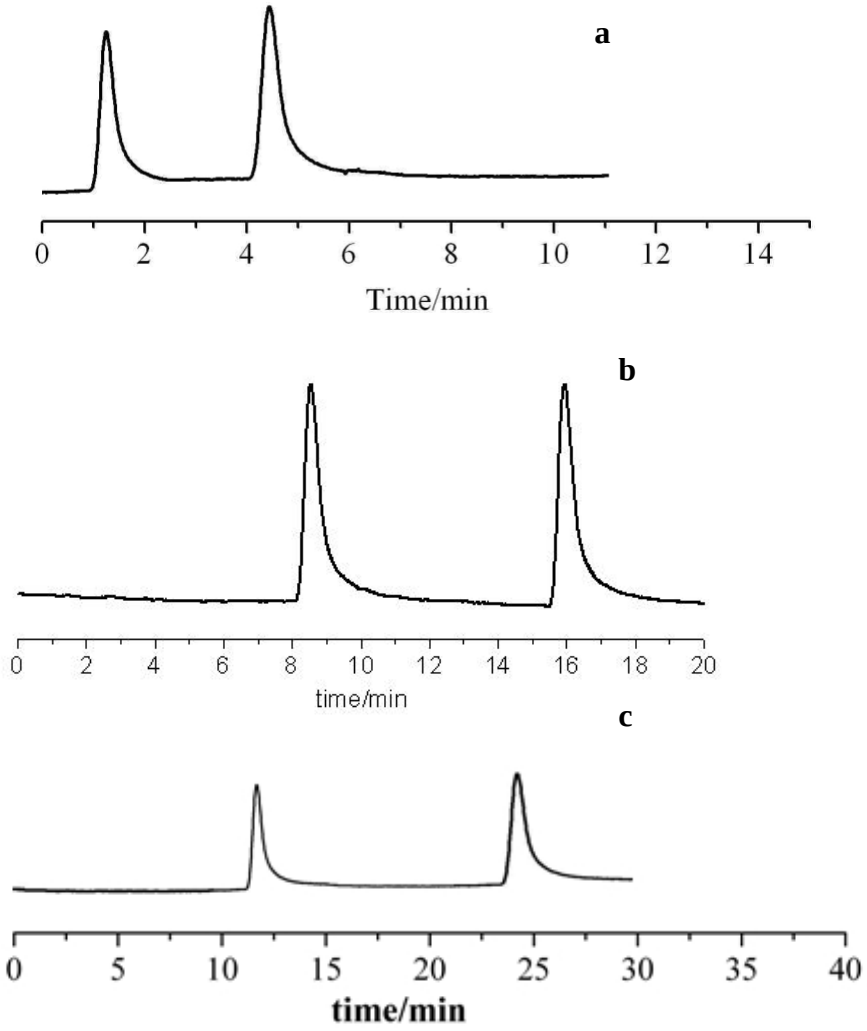


Figure 4.8 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different composition of HFIP/H_2O in the presence of 0.1 M NaCl: (a) 8/2, (b) 7/3, and (c) 6/4 (v/v). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection limit 254 nm; room temperature.

Table 4.3 Separation factor, resolution factor, theoretical plate numbers and height equivalent plate number at the chromatogram of the mixture of 2-naphthol and 4-nitroaniline under different compositions of HFIP/H_2O in the absence of 0.1 M NaCl

Mobile phase	separation factor (α)	Rs	N		HETP	
			2-naphthol	4-nitroaniline	2-naphthol	4-nitroaniline
HFIP/H_2O						
8/2	2.80	1.00	20	71	22781	6310
7/3	2.33	1.67	33	256	13781	1757
6/4	3.59	6.67	135	1664	3323	270

Table 4.4 Separation factor, resolution factor, theoretical plate numbers and height equivalent plate number at the chromatogram of the mixture of 2-naphthol and 4-nitroaniline under different mobile phase compositions and all mobile phase contained 0.1 M NaCl.

Mobile phase	separation factor (α)	Rs	N		HETP	
			2-naphthol	4-nitroaniline	2-naphthol	4-nitroaniline
AN/H_2O						
6/4	3.25	4.63	106	828	4253	543
4/6	1.94	4.65	410	2007	1096	224
2/8	1.86	5.14	690	2195	651	205
1-propanol/H_2O						
7/3	2.18	2.34	88	352	5136	1280
6/4	3.31	3.98	62	654	7218	688
2/8	2.29	4.71	271	1246	1658	361
2-propanol/H_2O						
4/6	3.16	3.02	40	343	11314	1312
2/8	2.35	7.53	818	2498	550	180
HFIP/H_2O						
8/2	5.86	4.25	36	529	12500	850
7/3	1.88	9.73	2202	7373	204	61
6/4	2.09	15.6	3600	15000	125	30

4.2 The separation of organic compounds using ionic liquids

The effect of different alkyl groups on imidazolium cation was studied. Three ionic liquids with different alkyl groups such as $EMIM^+Cl^-$, $BMIM^+Cl^-$ and $HMIM^+Cl^-$ were used to study this effect on the separation of organic compounds (2-naphthol and 4-nitroaniline). The chromatograms are shown in Figure 4.9-4.11.

Interestingly, low concentration of ionic liquids could separate the mixture of 2-naphthol and 4-nitroaniline in the elution order of 2-naphthol and 4-nitroaniline, respectively. The separation of mixture can explain by the microheterogeneities and preferential of mixed solvents of ionic liquids in aqueous solution occurred in a micro tube.

The concentration of ionic liquids was varied to improve the separation of mixture. Figure 4.12 shows the effects of concentration of ionic liquids on the capacity factor ($k`$) of 2-naphthol and 4-nitroaniline. From the results, it clearly illustrated that the elution time of these analytes increased dramatically with increasing the concentration of ionic liquids. Complete separation of mixture was achieved at the concentration 0.45 M for $EMIM^+Cl^-$, 0.30 M for $BMIM^+Cl^-$ and 0.35 M for $HMIM^+Cl^-$.

The concentration of ionic liquids remarkably affected the separation and elution time of analytes. Separation factor (a) of the mixture of 2-naphthol and 4-nitroaniline as a function of the concentration of ionic liquids is shown in Figure 4.13. From the results, the values of the separation factor (a) for $BMIM^+Cl^-$ slightly increased and the values of a for $HMIM^+Cl^-$ were almost constant at the concentration 0.35-0.50 M. It can suggest that the increase of the concentration of $BMIM^+Cl^-$ and $HMIM^+Cl^-$ affected the elution time of both 2-naphthol and 4-nitroaniline. However, the values of a for $EMIM^+Cl^-$ rapidly increased with the increase of the concentration $EMIM^+Cl^-$ and the values of $k`$ of 2-naphthol slightly increased but the values of $k`$ of 4-nitroaniline significantly increased with the increase of the concentration of $EMIM^+Cl^-$. The efficiency of this method was shown by the theoretical plate number (N) of ionic liquids with different concentrations as shown in Table 4.5. $BMIM^+Cl^-$ provided higher efficiency than $EMIM^+Cl^-$ and $HMIM^+Cl^-$.

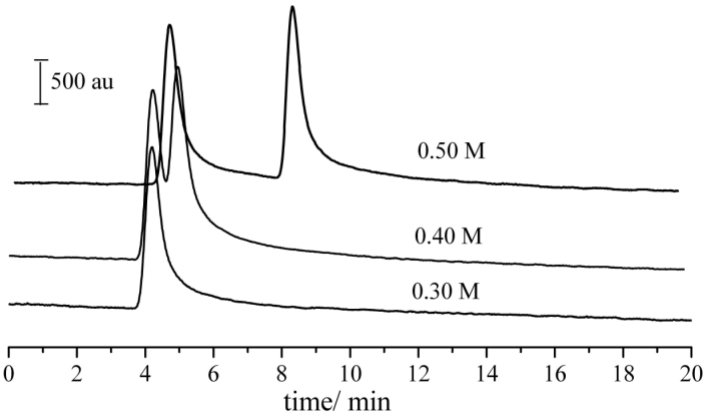


Figure 4.9 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different concentration of $EMIM^{+}Cl^{-}$: 0.30, 0.40 and 0.50 M. Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 254 nm; room temperature. Peaks: 2-naphthol: 1 and 4-nitroaniline: 2.

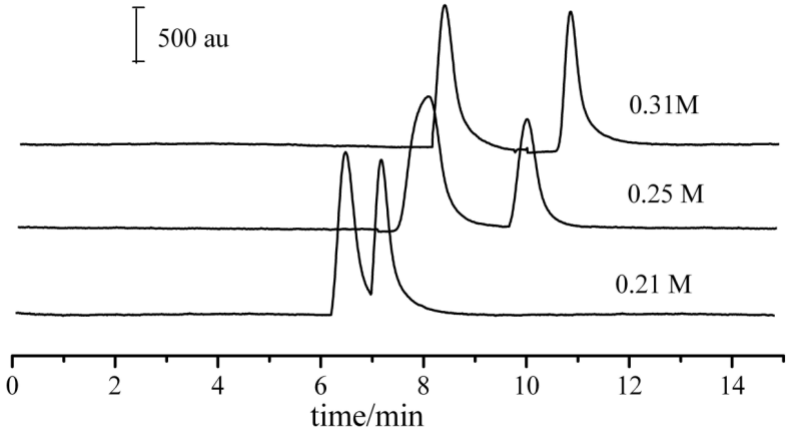


Figure 4.10 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different concentration of $BMIM^{+}Cl^{-}$: 0.21, 0.25 and 0.31 M. Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 254 nm; room temperature. Peaks: 2-naphthol: 1 and 4-nitroaniline: 2.

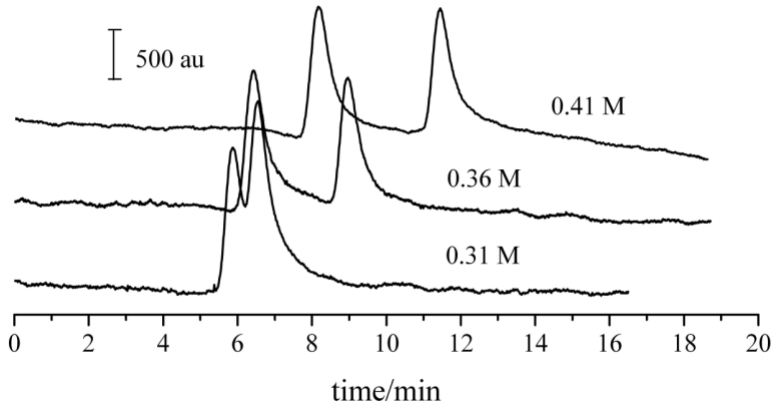


Figure 4.11 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different concentration of $HMIM^{+}Cl^{-}$: 0.31, 0.36 and 0.41 M. Chromatographic conditions: flow rate: 1 µL min^{-1}; detection: 254 nm; room temperature. Peaks: 2-naphthol: 1 and 4-nitroaniline: 2.

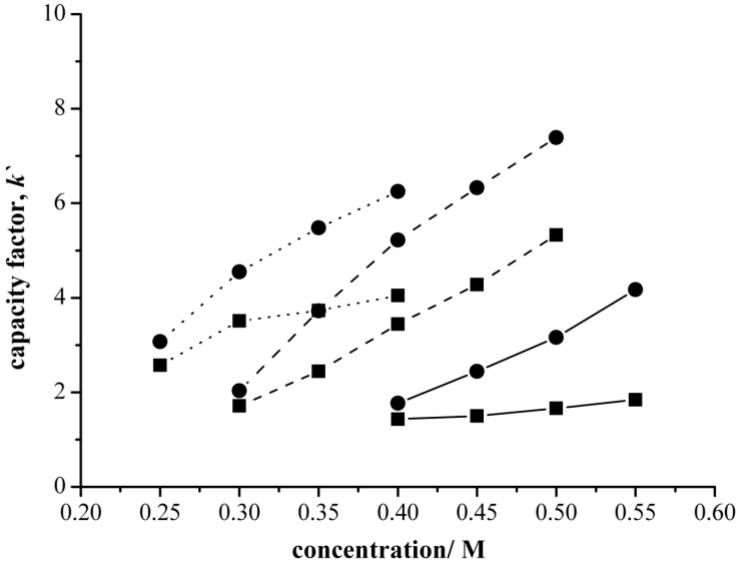


Figure 4.12 Capacity factor (*k*`) of 2-naphthol and 4-nitroaniline as a function of the concentration of ionic liquids of the mobile phase. Symbols: ■ = 2-naphthol, ● = 4-nitroaniline, ? = $EMIM^{+}Cl^{-}$, ····· = $BMIM^{+}Cl^{-}$, ----- = $HMIM^{+}Cl^{-}$.

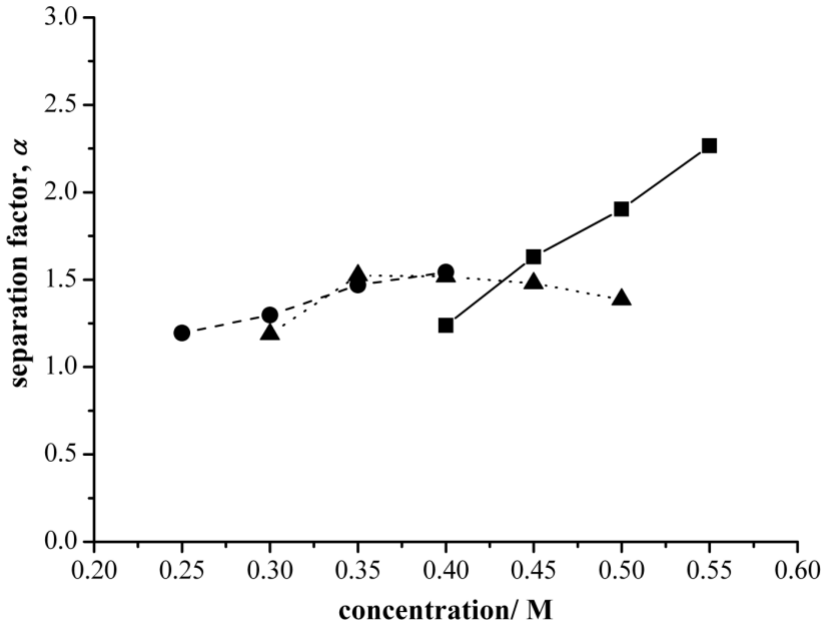


Figure 4.13 Separation factor (a) of the mixture of 2-naphthol and 4-nitroaniline as a function of the concentration of ionic liquids of the mobile phase. Symbols: ? ■? = $EMIM^+C\Gamma$, ---- ●---- = $BMIM^+C\Gamma$, ·····? ····· $HMIM^+C\Gamma$.

Table 4.5 Separation factor (a), theoretical plate number (N) and height equivalent theoretical plate (HETP) of 2-naphthol and 4-nitroaniline when $EMIM^+C\Gamma$, $BMIM^+C\Gamma$ and $HMIM^+C\Gamma$ used as mobile phase.

Ionic liquid	concentration	Theoretical plate nuber (N)		HETP (μm)	
	(M)	2-naphthol	4-nitroaniline	2-naphthol	4-nitroaniline
$EMIM^+C\Gamma$	0.40	278	392	1619	1148
	0.50	316	1181	1424	381
$BMIM^+C\Gamma$	0.25	676	876	666	514
	0.31	747	2015	602	223
$HMIM^+C\Gamma$	0.36	114	313	3947	1438
	0.41	125	409	3600	1100

Effect of $[BMIM]^+$ counterion

The effect of counterion was also studied. $[BMIM]^+$ with different counterions (Cl^-, Br^-, BF_4^-, $CF_3SO_3^-$) were used to investigate this effect. The separation of organic compounds is shown in Figure 4.14-4.16. The capacity factor (*k'*) and separation factor (α) of 2-naphthol and 4-nitroaniline were plotted as a function of concentration of ionic liquids as shown in Figure 4.17 and 4.18, respectively. The elution time of analytes increased when the concentration of ionic liquids increased and the trend of graph almost same. Considering the separation factor of mixture as a function of concentration of ionic liquids, for $[BMIM]^+$ Cl^- the separation factor values increased with the increasing the concentration while other anions the separation factor values almost constant.

The theoretical plate number of 2-naphthol and 4-nitroaniline were calculated as shown in Table 4.6.

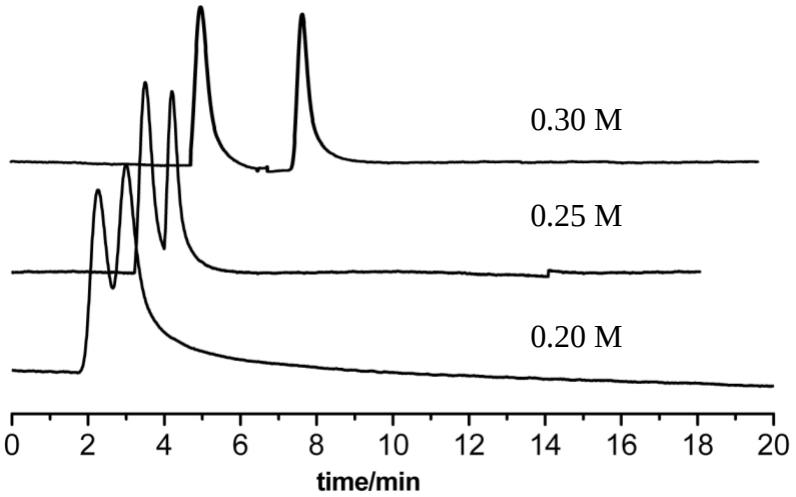


Figure 4.14 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different concentration of $BMIM^+Br^-$: 0.20, 0.25 and 0.30 M. Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 254 nm; room temperature. Peaks: 2-naphthol: 1 and 4-nitroaniline: 2.

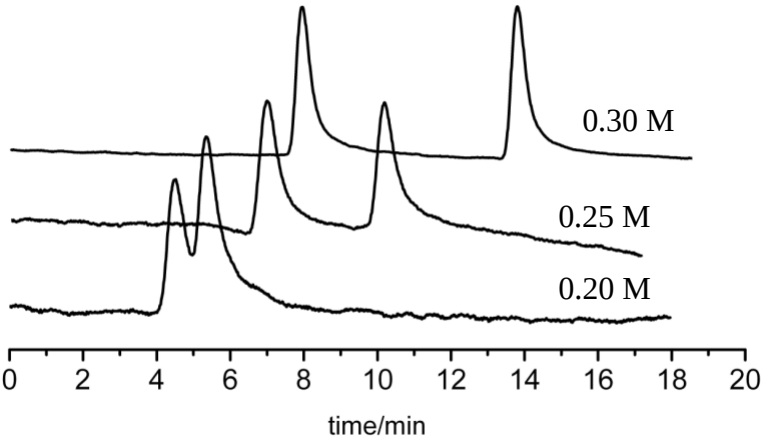


Figure 4.15 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different concentration of $BMIM^+BF_4^-$: 0.20, 0.25 and 0.30 M. Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 254 nm; room temperature. Peaks: 2-naphthol: 1 and 4-nitroaniline: 2.

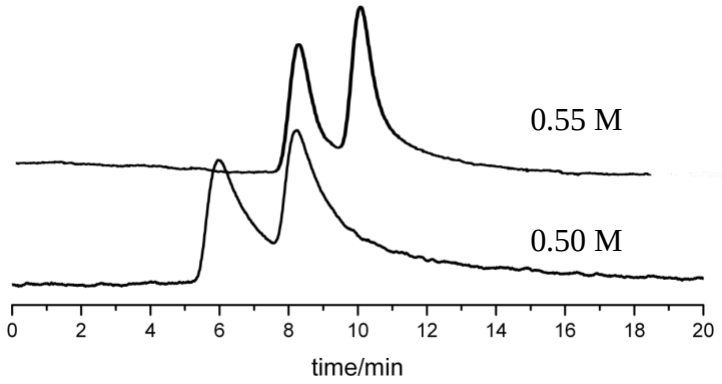


Figure 4.16 Chromatograms of mixture 2-naphthol and 4-nitroaniline with a mobile phase containing different concentration of $BMIM^+CF_3SO_3^-$: 0.50 and 0.55 M. Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 254 nm; room temperature. Peaks: 2-naphthol: 1 and 4-nitroaniline: 2.

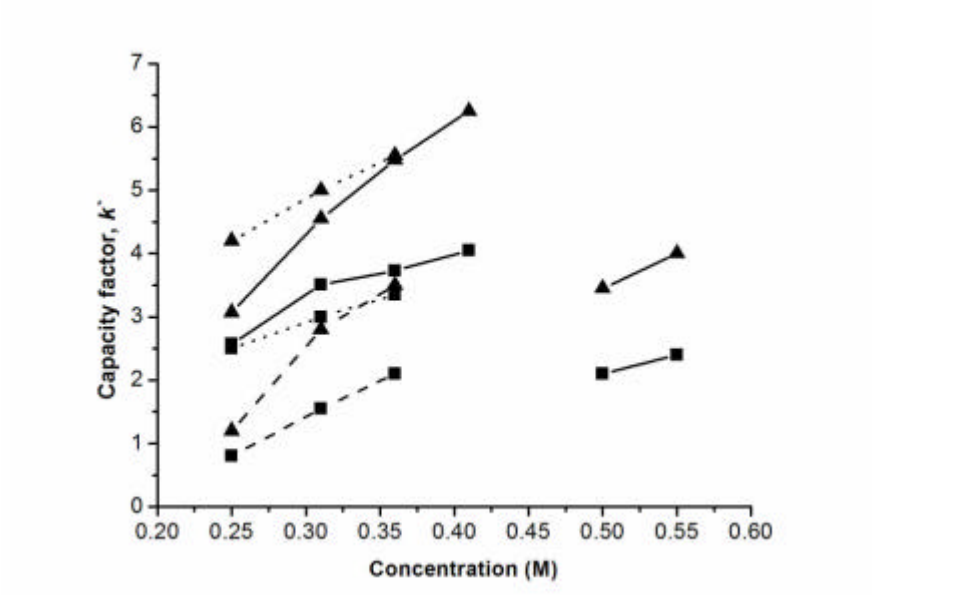


Figure 4.17 Capacity factor (*k*`) of 2-naphthol and 4-nitroaniline as a function of the concentration of ionic liquids of the mobile phase. Symbols: ■ = 2-naphthol, ▲ = 4-nitroaniline, ? = $BMIM^+Cl^-$, ----- = $BMIM^+Br^-$, ····· = $BMIM^+BF_4^-$, ? =$BMIM^+CF_3SO_3^-$.

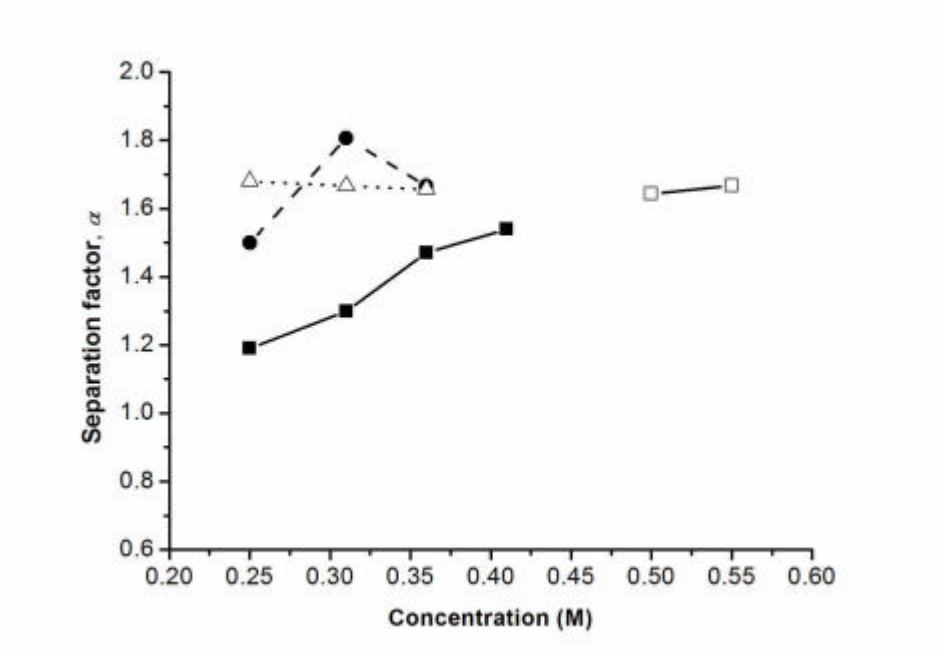


Figure 4.18 Separation factor (α) of the mixture of 2-naphthol and 4-nitroaniline as a function of the concentration of ionic liquids of the mobile phase. Symbols: ? ■? = $BMIM^+Cl^-$, ---●--- = $BMIM^+Br^-$, ···△··· = $BMIM^+BF_4^-$, ? □? =$BMIM^+CF_3SO_3^-$.

Table 4.6 Separation factor (α), theoretical plate number (N) and height equivalent theoretical plate (HETP) of 2-naphthol and 4-nitroaniline when $BMIM^+Br^-$, $BMIM^+BF_4^-$ and $BMIM^+CF_3SO_3^-$ used as mobile phase.

Ionic liquid	concentration	Theoretical plate nuber (N)		HETP (μm)	
	(M)	2-naphthol	4-nitroaniline	2-naphthol	4-nitroaniline
$BMIM^+Br^-$	0.25	676	876	666	514
	0.31	747	2015	602	223
$BMIM^+BF_4^-$	0.25	256	306	1758	1471
	0.30	286	411	1573	1095
$BMIM^+CF_3SO_3^-$	0.50	153	267	2941	1685
	0.55	139	468	3237	962

The E_T^N value, which is an empirical parameter of solvent polarity, was taken into account to elucidate the elution mechanism. The E_T^N values of the mixture of ionic liquid and water were measured at different ionic liquids (Table 4.7). The values of each ionic liquid were not significantly different. Therefore, the E_T^N value cannot explain the different results when using different ionic liquids.

Table 4.7 The wavelength of the maximum absorption band of DTP at 0.5 M of each ionic liquid.

Ionic liquids	**Wavelength (nm)**	$E_T(30)$	E_T^N
$BMIM^+Cl^-$	475	251.8	0.910
$BMIM^+Br^-$	478	250.2	0.899
$BMIM^+BF_4^-$	482	248.1	0.883
$BMIM^+CF_3SO_3^-$	480	249.2	0.890
$EMIM^+Cl^-$	472	253.4	0.922
$HMIM^+Cl^-$	476	251.3	0.906

4.3 The separation of organic compounds using ionic liquid and polymers

Figure 4.19 shows a chromatogram of phenols. Ionic liquid, $BMIM^+Cl^-$, separated the mixture at the concentration higher than 1.0 M. The elution orders were 2-naphthol, phenol, 4-chlorophenol and 4-nitrophenol, respectively. The elution time of compound was confirmed by injection of each compound separately. Less-polar compounds elute fast and acidic phenol elutes slowly last. The p*K*a values of the phenols are 9.46 (2-naphthol), 9.95 (phenol), 9.38 (4-chlorophenol), 7.08 (4-nitrophenol), respectively.[66] The p*K*a value of 2-naphthol is lower than that of phenol but it eluted before phenol. This suggests much importance of the hydrophobicity of the compounds in the present separation system. Water-soluble compounds are retained in water-phase on the surface of capillary tube and non-polar compounds are concentrated at the center of the capillary tube. This is why the above elution order was observed. Neutral organic compounds were successfully separated by just injection of compounds and through a flow of mobile phase by a micro-syringe pump. The separation factor for neighboring compounds eluted were 2.50, 1.17 and 1.28 from 2-naphthol to 4-nitrophenol, and the theoretical plate numbers were 332, 757, 1003 and 2460 for 2-naphthol, phenol, 4-chlorophenol and 4-nitroaniline, respectively.

The peak shapes, however, were tailed by a little due to that micro-solvent cluster did not so strongly assemble. To stabilize the ionic solvent clusters in the mixed solvent a neutral polymer, PVP, was added a little into the mobile phase solvent. Since ionic liquid molecules interact with polymer strongly, it is expected that ionic liquid molecules solvate to the polymer, leading the growth of ionic liquid solvent clusters in the mixed solvents. At low concentration (0.01 M) of PVP phenols were not separated sufficiently, but at concentration 0.10 M of PVP phenols were separated well and sharp peaks were observed (Fig. 4.20). Addition of PVP stabilizes the micro-solvent cluster and separation was improved significantly. The separation factors and theoretical plate numbers are summarized in Table 4.8.

Both ionic liquid and PVP are essential components for the separation of organic compounds under the present method, but the concentration of ionic liquid is more important to get a sufficient separation of the compounds. Figure 4.21 shows the effects of $BMIM^+Cl^-$ on the separation of the organic compounds. Much lower concentration of $BMIM^+Cl^-$ could not separate them and $BMIM^+Cl^-$ higher than 0.8 M was needed. Ionic liquid separates organic compounds and PVP improves the separation, spatially theoretical plate numbers (Table 4.8). To know the effect of PVP in more detail for the separation of phenols, the capacity factor (k') and the height

equivalent theoretical plate (HETP) of each compound were plotted against the concentration of PVP (Fig. 4.22). The retention time of analytes, especially phenol, 4-chlorophenol, and 4-nitrophenol increased at higher concentration of PVP and the HETP decreased when PVP was 0.01 M to 0.1 M, and then was constant at higher than 0.10 M. The decreased HETP and the increased *k'* values suggest a growth of ionic liquid solvent clusters around PVP and an increased trap of the compounds in the clusters.

LAXS data suggest the formation of micro-solvent cluster formation in the mixed solvent. Ukei *et al*[67,68] reported the occurrence of a phase separation of ionic liquid when biopolymers were dissolved in the ionic liquids, showing a cloud temperature. Thus PVP enhances the aggregation of ionic liquid around the polymer in the mixed aqueous solution. The increased micro-solvent clusters of the ionic liquid increase theoretical plate number and separation factor. Aromatic compounds are concentrated in the ionic liquid clusters around PVP and polar or ionic compounds are concentrated in the water phase around the surface of capillary tube. Ionic compounds therefore elute slowly. In conclusion, we show a new type of separation method based on "micro-solvent cluster extraction" using ionic the liquid and PVP in aqueous solution.

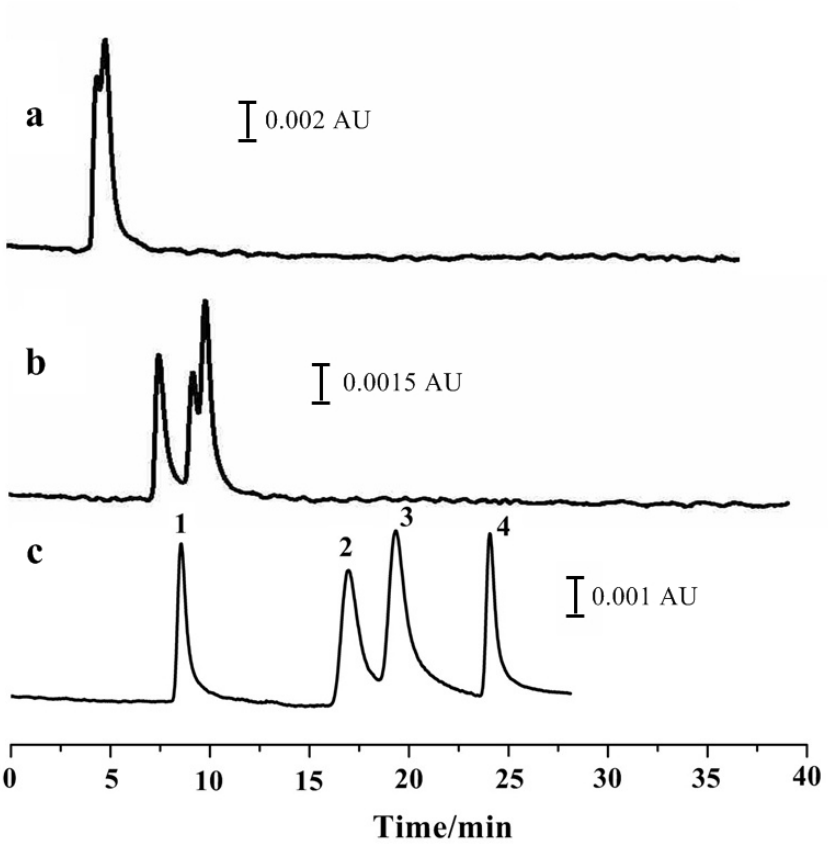


Figure 4.19 Chromatograms of 2.5 mM phenol derivatives with a mobile phase containing different concentrations of $BMIM^{+}Cl^{-}$: 0.5 M (a), 0.8 M (b), and 1.0 M (c). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 223 nm; room temperature. Peaks: 2-naphthol (1), phenol (2), 4-chlorophenol (3), and 4-nitrophenol (4).

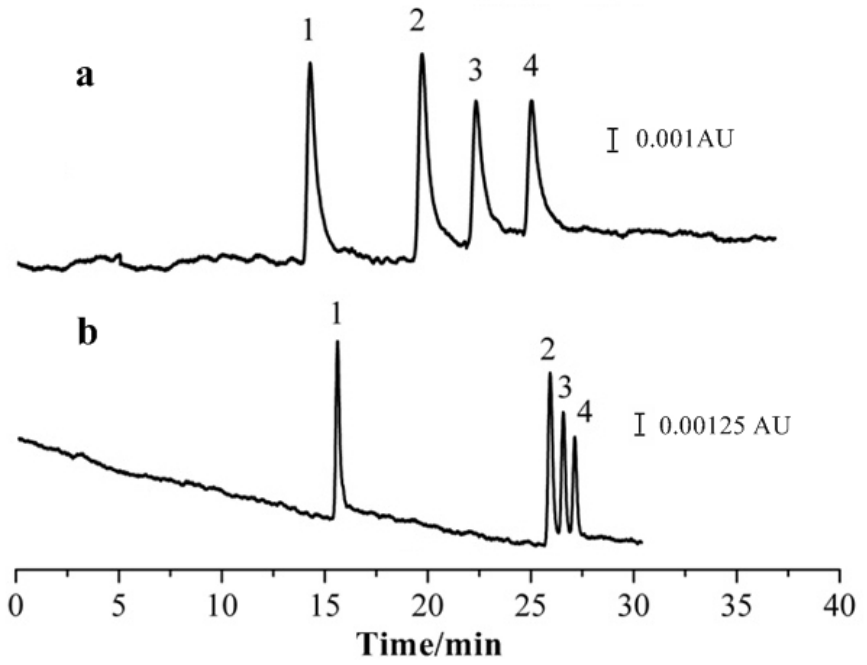


Figure 4.20 Chromatograms of 2.5 mM phenol derivatives with a mobile phase 1.0 M $BMIM^{+}Cl^{-}$ containing different concentrations of PVP : 0.01 M (a) and 0.1 M (b). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 223 nm; room temperature. Peaks: 2-naphthol (1), phenol (2), 4-chlorophenol (3), and 4-nitrophenol (4).

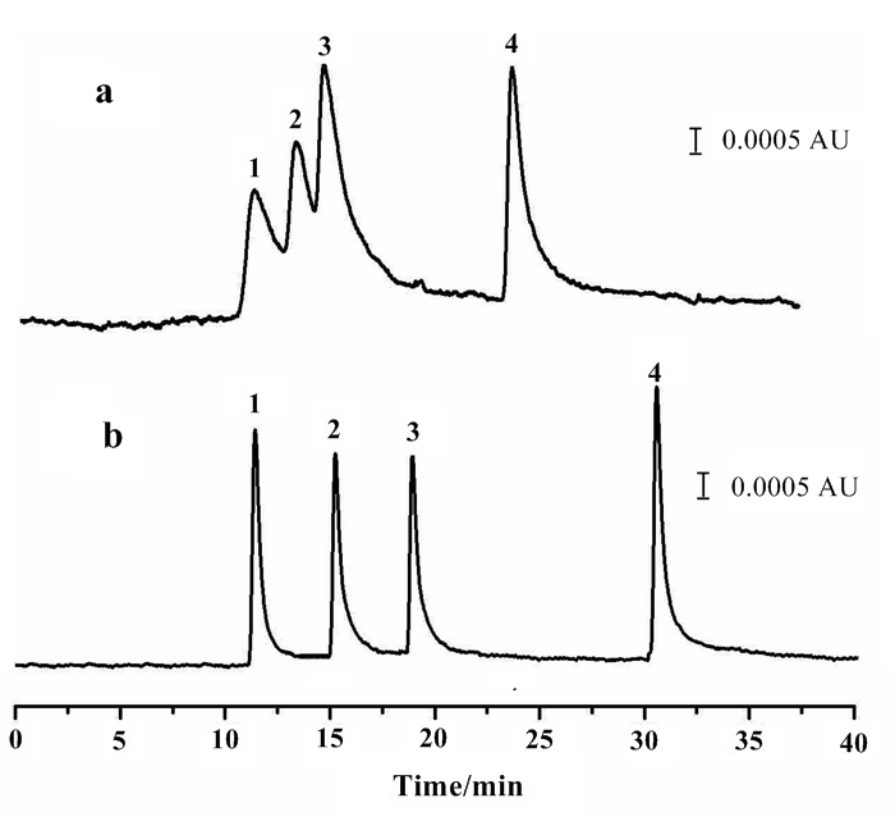


Figure 4.21 Chromatograms of 2.5 mM phenol derivatives with a mobile phase containing 0.5 M $BMIM^+Cl^-$ and 0.01 M PVP (a), and 0.8 M $BMIM^+Cl^-$ and 0.01 M PVP (b). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 223 nm; room temperature. Peaks: 2-naphthol (1), phenol (2), 4-chlorophenol (3), and 1,3,5-naphthalenetrisulfonic acid (4).

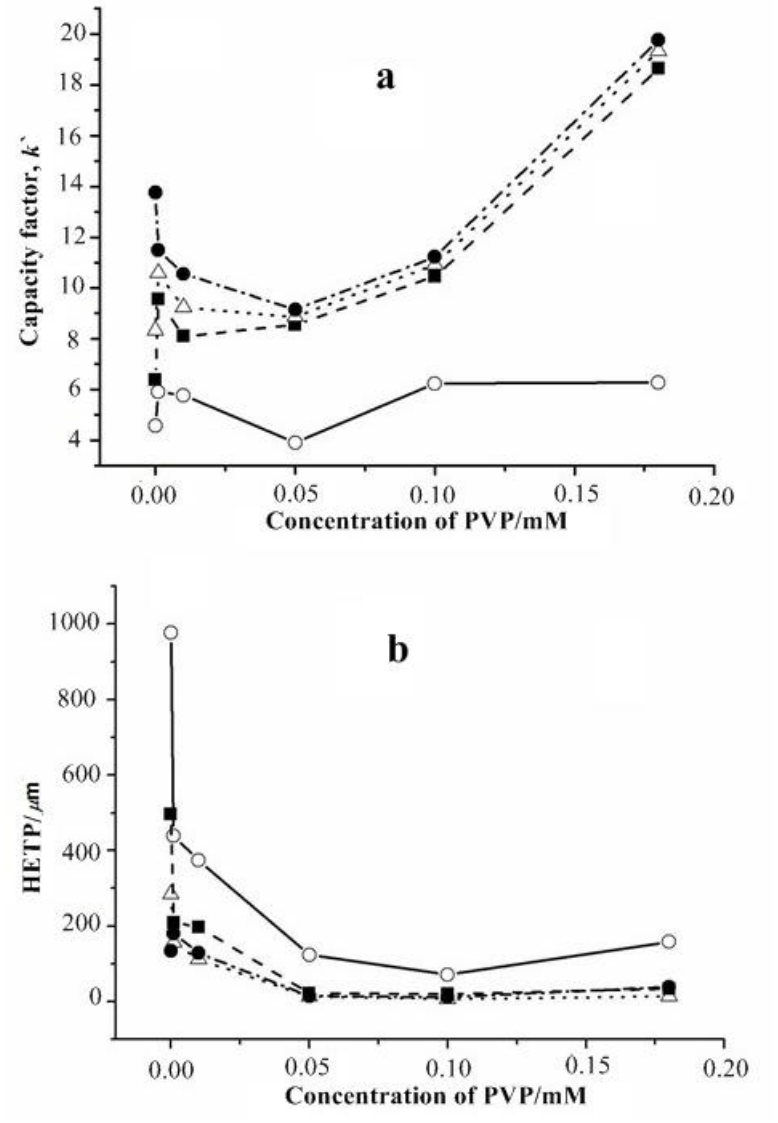


Figure 4.22 Effects of PVP concentrations on capacity factor (*k`*) (a), and height equivalent theoretical plate (HETP) (b) of phenol derivatives at the concentration of $BMIM^{+}Cl^{-}$ 1.0 M. Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 223 nm; room temperature. *Symbols:* 2-naphthol: ○, phenol: ■, 4-chlorophenol: △, 4-nitrophenol: ●.

Table 4.8 Separation factor (α), theoretical plate number (N) and height equivalent theoretical plate (HETP) of phenols when $BMIM^{+}Cl^{-}$ 1.0 M and PVP concentration was varied used as mobile phases

compound	**Concentration of PVP (M)**	**Separation factor**	**N**	**HETP (mm)**
2-naphthol	0	2.50	332	1355
	0.01	1.40	1204	374
	0.10	1.68	6320	71
phenol	0	1.17	757	594
	0.01	1.14	2281	197
	0.10	1.04	22907	20
4-chlorophenol	0	1.28	1003	449
	0.01	1.14	4039	111
	0.10	1.03	63465	7
4-nitrophenol	0		2460	183
	0.01		3505	128
	0.10		37184	12

4.4 The separation of organic compounds using polymer ionic liquids

In this research, the polymer ionic liquid has been used to separate the model compounds. The mixture could separate well and gave sharp peak. Figure 4.23 shows a chromatogram of phenols. Ionic liquid, $C_{10}MIM^{+}Cl^{-}$, separated the mixture at the concentration 0.5 M. The elution orders were 2-naphthol, phenol, 4-chlorophenol and 4-nitrophenol, respectively. Table 4.9 shows the chromatographic parameters at different concentration of $C_{10}MIM^{+}Cl^{-}$.

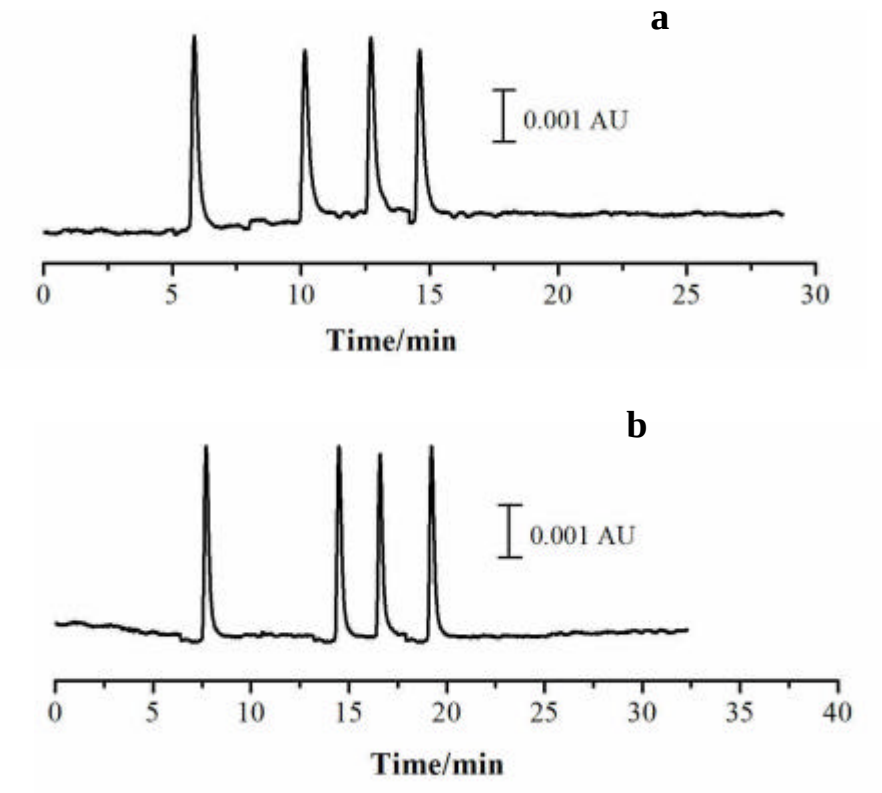


Figure 4.23 Chromatograms of 2.5 mM phenol derivatives with a mobile phase containing 0.5 M $C_{10}MIM^{+}Cl^{-}$ (a), and 0.8 M $C_{10}MIM^{+}Cl^{-}$ (b). Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 223 nm; room temperature. Peaks: 2-naphthol (1), phenol (2), 4-chlorophenol (3), 4-nitrophenol (4).

The separation of soluble water compound; 1,3,5-NTS, was eluted slowly. It suggests that the micro-phase separation mechanism of capillary tube occurs as the proposed mechanism.

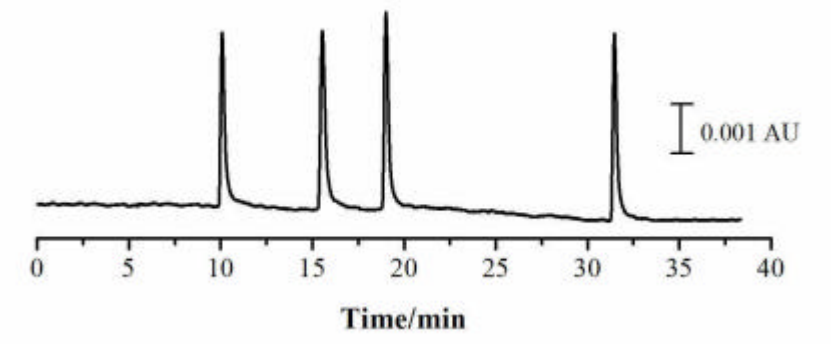


Figure 4.24 Chromatograms of 2.5 mM phenol derivatives with a mobile phase containing 0.5 M $C_{10}MIM^{+}Cl^{-}$. Chromatographic conditions: flow rate: 1 μL min^{-1}; detection: 223 nm; room temperature. Peaks: 2-naphthol (1), phenol (2), 4-chlorophenol (3), and 1,3,5-naphthalenetrisulfonic acid (4).

Table 4.9 Separation factor (α), theoretical plate number (N) and height equivalent theoretical plate (HETP) of phenols at different concentration of $C_{10}MIM^{+}Cl^{-}$.

compound	**Concentration (M)**	**Separation factor**	**N**	**HETP (μm)**
2-naphthol	0.10	2.09	576	781
	0.25	2.29	992	454
	0.50	1.66	3059	147
phenol	0.10	1.28	2196	205
	0.25	1.19	3557	127
	0.50	1.21	3084	146
4-chlorophenol	0.10	1.19	2500	180
	0.25	1.18	3795	119
	0.50	1.21	5388	84
4-nitrophenol	0.10		5256	86
	0.25		8405	54
	0.50		15876	28

CHAPTER V

CONCLUSIONS

Utilizing the microheterogeneities, preferential salvation and salted-out phase separation in aqueous mixed solvents, such as acetonitrile, 1-propanol, 2-propanol, HFIP, ionic liquids, a new separation method has been proposed using an open capillary tube. Several model compounds have separated by the proposed system. For organic solvents, the separation power of the proposed method is due to salt enhanced micro-phase separation of the mobile phase between the capillary wall and the center of capillary tube.

The model compound have separated by using ionic liquids in water due to the formation of micro-solvent cluster formation of ionic liquid in the mixed solvent that was suggested by LAXS data. Ukei *et al*[67,68] reported the occurrence of a phase separation of ionic liquid when biopolymers were dissolved in the ionic liquids, showing a cloud temperature. Thus PVP enhances the aggregation of ionic liquid around the polymer in the mixed aqueous solution**.** The increased micro-solvent clusters of the ionic liquid increase theoretical plate number and separation factor. Aromatic compounds are concentrated in the ionic liquid clusters around PVP and polar or ionic compounds are concentrated in the water phase around the surface of capillary tube. Ionic compounds therefore elute slowly. A new type of separation method based on "micro-solvent cluster extraction" using ionic the liquid and PVP in aqueous solution is given in Figure 5.1.

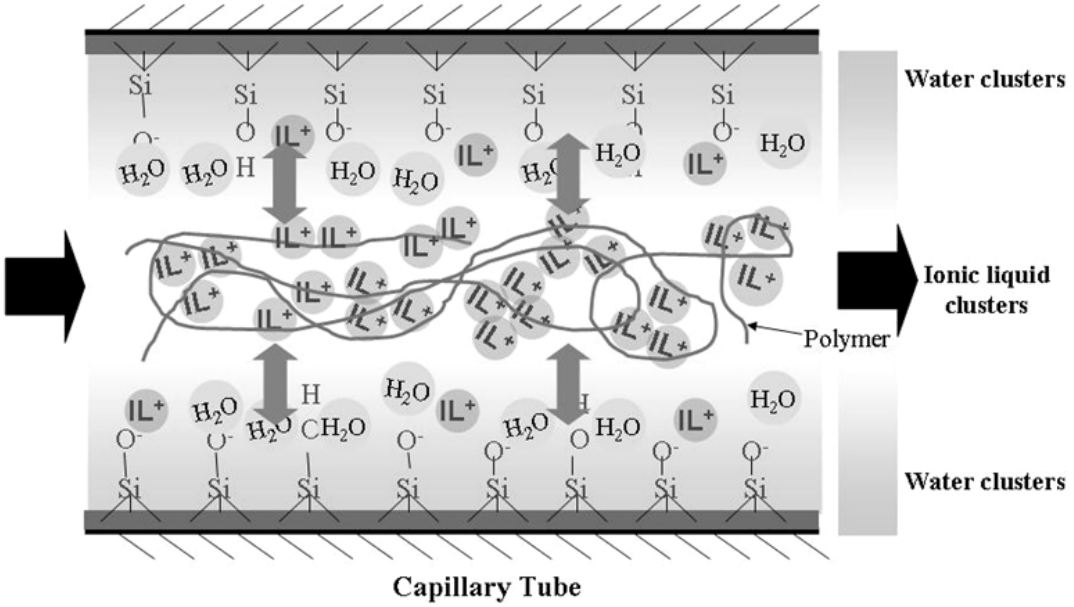


Figure 5.1 Micro-solvent cluster extraction mechanism using ionic liquid and polymer.

REFERENCES

1. M. Tabata, Y. G. Wu, T. Charoenraks, S. S. Samaratunga, *Bull. Chem. Soc. Jpn*. **2006,** *79*, 1742.
2. T. Takamuku, M. Tabata, A. Yamaguchi, J. Nishimoto, M. Kumamoto, H. Wakita, T. Yamaguchi, *J. Phys. Chem. B* **1998**, *102*, 8880.
3. Y. G. Wu, M. Tabata, T. Takamuku, *J. Mol. Liquid* **2001**, 94, 273.
4. Y. G. Wu, M. Tabata, T. Takamuku, *Talanta* **2001**, *54*, 69.
5. Y. G. Wu, M. Tabata, *J. Solution Chem.* **1998**, *33*, 777.
6. Y. G. Wu, M. Tabata, T. Takamuku, *J. Solution Chem.* **2002**, *31*, 381.
7. T. Takamuku, A. Yamaguchi, D. Matsuo, M. Tabata, M. Kumamoto, J. Nishimoto, K. Yoshida, T. Yamaguchi, M. Nagao, T. Otomo, T. Adachi, *J. Phys. Chem. B* **2001**, *105*, 6236.
8. T. Takamuku, A. Yamaguchi, D. Matsuo, M. Tabata, T. Yamaguchi, T. Otomo, T. Adachi, *J. Phys. Chem. B* **2001**, 105, 10101.
9. G. A. Baker, S. N. Baker, S. Pandey, F. V. Bright, *Analyst* [London] **2005**, *130*, 800.
10. S. A. Shamsi, N. D. Danielson, *J. Sep. Sci.* **2007**, 30, 1729.

11. M. J. E. Golay, *Anal. Chem*. **1968**, *40*, 382.

12. J. H. Knox, M. T. Gilbert, *J. Chromatogr.* **1979**, *186*, 405.

13. G. Liu, N. M. Djordjevic, F. Erni, *J. Chromatogr. A* **1992**, *592*, 239.

14. S. R. Muller, W. Simon, H. M. Widmor, K. Grolimund, G. Schomburg, P. Kolla, *Anal. Chem*. **1989**, *61*, 2747.

15. R. swart, J. C. Kraak, H. Poppe, *Trends Anal. Chem*. **1997**, *16*, 332.

16. L. M. Nyholm, K. E. Markides, *J. Chromatogr. A* **1998**, *813*, 11.

17. Y. Nakano, S. Kitagawa, K. Miyabe, T. Tsuda, *Anal. Sci.* **2005**, *21*, 1167.

18. H. Li, Y. Chen, Z. Zeng, C. Xie, X. Yang, *Anal. Sci.* **2005**, *21*, 717.

19. J. J. Pesek, M. T. Matyska, *J. Chromatogr. A* **2000**, *887*, 31.

20. J. L. Anderson, D. W. Armstrong, G.-T. Wei, *Anal. Chem.* **2006**, *78*, 2892.

21. http://ilthermo.boulder.nist.gov/ILthermo/mainmenu.uix

22. M. Cruz-Vera, R. Lucena, S. Cardenas, M. Valcarcel, *J. Chromatogr. A* **2008,** *1202*, 1.

23. E. Aguilera-Herrador, R. Lucena, S. Cardenas, M. Valcarcel, *J. Chromatogr. A* **2008,** *1201*, 106.

24. J. G. Huddleston, H. D. Willauer, R. P. Swatloski, A. E. Visser, R. D. Rogers, *Chem. Commun.* **1998,** 1756.

25. A. E. Visser, R. P. Swatloski, W. M. Reichert, R. Mayton, S. Sheff, A. Wierzbicki, J. H. Davis, Jr. and R. D. Rogers, *Chem. Commun.* **2001,** 135.

26. J.-F. Liu, G.-B. Jiang, Y.-G. Chi, Y.-Q. Cai, Q.-X. Zhou, J.-T. Hu, *Anal. Chem.* **2003,** *75*, 5870.

27. M. C. Buzzeo, R. G. Evans, R. G. Compton, *ChemPhysChem.* **2004,** *5*, 1106.

28. I. M. AlNashef, M. L. Leonard, M. A. Matthews, J. W. Weidner, *Ind. Eng. Chem. Res.* **2002,** *41*, 4475.

29. M. C. Buzzeo, C. Hardacre, R. G. Compton, *Anal. Chem.* **2004,** *16*, 66.

30. R. Wang, T. Okajima, F. Kitamura., T. Ohsaka, *Electroanalysis* **2004,** *16*, 66.

31. D. W. Armstrong, L.-K. Zhang, L. He, M. L. Gross, *Anal. Chem.* **2001,** *73*, 3679.

32. M. Mank, B. Stahl, G. Boehm, *Anal. Chem.* **2004,** *76*, 2936.

33. J. A. Levisky, R. A. Wilson, C. L. Hussey, *Inorg. Chem.* **1982,** *21*, 1263.

34. W. Lu, A. G. Fadeev, B. Qi, E. Smela, B. R. Mattes, J. Ding, G. M. Spinks, J. Mazurkiewicz, D. Zhou, G. G. Wallace, D. R. MacFarlane, S. A. Forsyth, M. Forsyth, *Science,* **2002,** *297*, 983-987.

35. R. A. Osteryoung, *General Review: Molten salt Chemistry: An Introduction and Selected Applications* **1987,** *202*, 329-364.

36. M. Yoshizawa, A. Narita, H. Ohno, *Aust. J. Chem.* **2004,** *57,* 139.

37. S. A. Shamsi, N. D. Danielson, *J. Sep. Sci.* **2007,** *30,* 1729.

38. G. Inoune, Y. Iwai, M. Yasutake, K. Honda, Y. Arai, *Fluid Phase Equil.* **2007,** *251,* 17-23.

39. Q. Zhou, L.-S. Wang, *J. Chem. Eng. Data* **2006,** *51,* 1698-1701.

40. Q. Zhou, L.-S. Wang, J.-S. Wu, M.-Y. Li, *J. Chem. Eng. Data* **2007,** *52,* 131.

41. C. F. Poole, K. G. Furton, B. R. Kerstern, *J. Chromatogr. Sci.* **1986,** *24,* 400.

42. F. Pacholec, H. T. Butter, C. F. Poole, *Anal. Chem.* **1982,** *54,* 1938.

43. C. F. Poole, H. T. Butter, M. E. Coddens, S. C. Dhanesar, F. Pacholec, *J. Chromatogr.* **1984,** *289,* 299.

44. J. L. Anderson, D. W. Armstrong, *Anal. Chem.* **2005,** *77,* 6453.

45. G. R. Lambertus, J. A. Crank, M. E. Mc Guigan, S. Kendler, D. W. Armstrong, R. P. Sacks, *J. Chromatogr. A* **2006,** *1135,* 230.

46. P. H. Shetty, S. K. Poole, C. F. Pool, *Anal. Chim. Acta.* **1990,** *236,* 51.

47. Y. Sun, B. Cabovska, C. E. Evans, T. H. Ridgway, A. M. Stalcup, *Anal. Bioanal. Chem.* **2005,** *382,* 728.

48. Y. Sun, A. M. Stalcup, *J. Chromatogr. A* **2006,** *1126,* 276.

49. Y. Polyakova, Y. Koo, K. H. Row, *Biotech. Bioprocess Eng.* **2006,** *11,* 1.

50. R. Kaliszan, M. P. Marszall, M. J. Markuszewski, T. Baczek, J. Pernak, *J. Chromatogr. A* **2004,** *1030,* 263.

51. Y. Polyakova, Y. Jin, J. Zheng, K. W. Row, *J. Liq. Chromatogr. Relat. Technol.*, **2006,** *29,* 1687.

52. J. Zheng, K. H. Row, *Am. J. Appl. Sci.* **2006,** *3,* 2160.

53. F. Tang, L. Tao, X. Luo, L. Ding, M. Guo, L. Nie, S. Yao, *J. Chromatogr. A* **2006,** *1125,* 182.

54. M. Vaher, M. Koel, M. Kaljurand, *Chromatographia* **2001,** *53,* S302.

55. M. Vaher, M. Koel, M. Kaljurand, *Electropheresis* **2002,** *23,* 426.

56. E. G. Yanes, S. R. Gratz, M. J. Balwin, S. E. Robison, A. M. Stalcup, *Anal. Chem.* **2001,** *73,* 3838.

57. T.-F. Jiang, Y.-L. Gu, B. Liang, J.-B. Li. Y.-P. Shi, Q.-Y. Ou, *Anal. Chim. Acta* **2003,** *479,* 249.

58. W. Qin, S. F. Y. Li, *Analyst* **2003,** *128,* 37.

59. S. M. Mwongela, A. Numan, N. L. Gill., R. A. Agbaria, I. M. Warner, *Anal. Chem.* **2003,** *75,* 6089.

60. S. A. Rizvi, S. A. Shamsi, *Anal. Chem.* **2006,** *78,* 7061.

61. V. P. Schnee, G. A. Baker, E. Rauk, C. P. Palmer, *Electrophoresis* **2006,** *27,*

4141.

62. R. Keller, J.-M. Mermet, M. Otto, H. M. Widmer, *"Analytical Chemistry"* Wiley-VCH. **1998,** 157-213.

63. G. D. Christian, *"Analytical Chemistry"* 4th Edition, John Wiley & Sons. **1986,** 442-479.

64. M. Yasuda, T. Sonda, T. Hiraoka, A. Horita, M. Tabata, *Anal. Sci.* **2003,** *19,* 1637.

65. M. Fioroni, K. Burger, A. E. Mark, D. Roccatano, *J. Phys. Chem. B* **2001**, 105, 10967.

66. A. E. Martell and R. M. Smith, *"Critical Stability Constants"* **1977**, *3,* Other Organic Ligands, *Plenum Press,* New York and London.

67. T, Ueki, M. Watanabe, *Langmuir* **2007**, *23,* 988.

68. T. Ueki, T. Karino, Y. Kobayashi, M. Shibayama, M. Watanabe, *J. Phys Chem. B* **2007**, *111*, 4750.